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Impact of carbohydrate reduced nutrition in septic patients on ICU - study protocol for a prospective randomized controlled trial

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Abstract

Introduction: Sepsis is defined as detrimental immune response to an infection. This overwhelming reaction often abolishes a normal reconstitution of the immune cell homeostasis that in turn increases the risk for further complications. Recent studies revealed a favorable impact of ketone bodies on resolution of inflammation. Thus, a ketogenic diet may provide an easy-to-apply and cost-effective treatment option potentially alleviating sepsis-evoked harm. This study is designed to assess the feasibility, efficiency and safety of a ketogenic diet in septic patients.

Methods and analysis: This monocentric study is a randomized, controlled, and open-label trial, conducted on an intensive care unit of a German university hospital. As intervention enteral nutrition with reduced amount of carbohydrates (ketogenic) or standard enteral nutrition (control) is applied. The primary endpoint is the detection of ketone bodies in patients' blood and urine samples. As secondary endpoints the impact on important safety relevant issues (e.g. glucose metabolism, lactate serum concentration, incidence of metabolic acidosis, thyroid function, and 30-day mortality) and the effect on the immune system is analysed.

Ethics and dissemination: The study has received the following approvals: Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6557-BR). Results will be made available to critical care survivors, their caregivers, the funders, the critical care societies and other researchers by publication in a peer-reviewed journal.

Trial registration: German trial register (DRKS.de) identifier is DRKS00017710 pre-registered on August 2nd, 2019; Universal Trial Number (UTN) is U1111-1237-2493

Article summary

Strengths and limitations of this study

- This is the first randomized controlled trial investigating whether an enteral nutrition with low amounts of carbohydrates is sufficient to induce ketone bodies in critically ill patients suffering from sepsis.
- This trial contributes to assess the feasibility and safety of a ketogenic response in septic patients induced by carbohydrate-reduced enteral nutrition on the intensive care unit.
- The secondary outcomes of this study will provide a first insight on the immunological response of septic patients to a ketogenic diet.
- Despite the prospective randomized controlled study design the lack of blinding is an immanent limitation within this study.

Keywords: Sepsis, low carb, ketogenic diet, carbohydrates, nutrition, inflammation

Introduction

Sepsis is a life-threatening condition characterized by a global dysregulation of the immune system: hyperinflammatory reactions, mostly mounted by innate immune cells and immunoparalysis of adaptive immune cells can occur in an unpredictable time course, sequentially or even simultaneously.[1 2 3]

Despite intensive research efforts during the last decade, mortality rates of sepsis still range around 30-50%, and causal therapies reconstituting immune homeostasis are not available so far.[4] In this situation, the impact of nutrition could gain importance, as metabolism has emerged as a major guiding force of immune cell functions.[5]

According to the ESPEN guideline on clinical nutrition in the ICU, patients receive an enteral nutrition consisting of 1,3g of protein/kg body weight/day, 1,5g of lipids/kg body weight/day. Carbohydrate administration in the range of 4-5mg/kg body weight/minute is recommended, and insulin should be administered at blood glucose levels >180mg/dl.[6] This regimen might now be reconsidered as recent experimental studies revealed that high intake of carbohydrates and consecutive secretion of insulin induces pro-inflammatory reactions of innate immune cells.[7] In line with these findings, a number of convincing studies have recently shown that reducing carbohydrate intake significantly stabilizes immune cell homeostasis and improves survival after systemic bacterial infection.[8 9 10] In these studies, the total amount of carbohydrates is reduced to approximately 10%, whereas protein amounts are kept constant and fat amounts are increased.[11 12] The reduced availability of glucose results in increase of fatty acid oxidation with subsequent synthesis of ketone bodies to cover the body's energy demand and to generate sufficient amounts of ATP.[13] This evolutionary conserved mechanism results in the synthesis of beta-hydroxybutyrate (BHB).[14] However, it becomes increasingly clear that BHB also functions as a signalling molecule by

affecting gene expression via epigenetic alterations, protein modifications, and G-Protein-coupled signaling.[15 16] In recent animal studies, BHB displayed strong anti-inflammatory effects by inhibiting the NLRP3 inflammasome and reducing proinflammatory cytokine secretion of innate immune cells, thus contributing to immune cell homeostasis.[14 16 17 18]

Ketogenic/low carb diets are an established clinical tool in patients suffering from epilepsy. Here, they significantly reduce seizure frequencies without displaying significant adverse effects.[19 20] Also, ketogenic/low carb nutritional regimes have recently been investigated in clinical studies enrolling overweight patients with Type II Diabetes[21] and patients suffering from Glioblastoma.[22] These studies reported no adverse side effects, providing additional evidence that ketogenic/low carb diets are feasible and safe.

In this prospective, randomized controlled trial, we want to assess feasibility and safety of a ketogenic diet in ICU patients suffering from sepsis. Moreover, we will investigate whether enteral administration of a low carb/ketogenic diet induces detectable levels of ketone bodies in septic patients, and whether these ketones are able to modulate immune responses during sepsis.

Methods and analysis

This study is a randomized, open-label trial comparing an interventional group supplied with a low-carb diet and a control group supplied with standard enteral nutrition.

Study population and general data acquisition

This study was reviewed and approved by the Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6657-BR) and registered in the German Clinical Trial Register (DRKS00017710; UTN: U1111-1237-2493) prior to the inclusion of the first study patient. The study will be conducted in accordance with the Declaration of Helsinki and German laws and regulations. All patients are admitted to the intensive care unit (ICU) of University Hospital Knappschaftskrankenhaus Bochum and are recruited from January 2020 (first patient in on January 22nd, 2020) up to February 2021. Patients are considered eligible if study enrolment is completed within 36h after diagnosis of sepsis according to the current Sepsis-3 definition.[23]

Inclusion criteria are age ≥ 18 years, written informed consent of the patient or their guardian, study enrolment within 36 hours after diagnosis of sepsis and mechanical ventilation for less than 72 hours on study inclusion. Exclusion criteria are pregnancy or lactation, haemoglobin concentration < 8g/dl, insulin-dependent diabetes, severe and persistently health-compromising metabolic disorders or autoimmune diseases, severe liver dysfunction or liver failure, refractory metabolic acidosis, invasive ventilation >72h, diagnosis of sepsis >36h at study enrolment and contraindications against an enteral nutrition.

After randomization, patient data collected are depersonalized via pseudonymization. All pseudonymized and deidentified clinical, biometrical and demographic data will be entered into an offline password-protected study database for later analysis. This dataset will include pre-existing illnesses, frequently used

scores such as the Simplified Acute Physiology Score II (SAPS II) or the Sepsis-related Organ Failure Assessment Score (SOFA), Body Mass Index (BMI), need and duration of renal replacement therapy, ventilator configurations, Horowitz-Index (ratio of PaO2/FiO2), vital parameters (e.g. heart rate, blood pressure, peripheral saturation), medications, amount and dosage of vasopressors and blood laboratory parameters.

Patient and public involvement

Patients were not involved in the development of the research question, outcome measures or study design.

Sample size calculation

In this randomized-controlled study, a total of 40 patients, i.e. 20 patients in the intervention group and 20 patients in the control group, will be enrolled. Based on available data on ketogenic diet regimes for healthy individuals[11] and our estimation of a clinical reasonable effect size, we assume an effect size (Cohen's d) between 1.34 and 2.14 as appropriate. Subsequently, we conducted sample size calculations with varying effect sizes between 1.34 and 2.14 at a level of significance of α=0.05. Based on these calculations, considering the most conservative effect size of 1.34 and assuming a drop-out-rate of 25% as a safety margin, a total sample size of n = 40 (n = 20:20) presents as adequate to achieve a power of 95% (figure 1)

Study design

The total duration of the study is planned for 18 months. It will take 12 months for recruitment of patients and collection of data. The last 6 months are scheduled for analyses. An individual study duration of 14 days is scheduled for each patient (figure

2). This includes study education and randomization (30 minutes), data collection, intervention with accompanying data collection in both interventional and control group (14 days). End of study is reached on day 14 (along with end of intervention) or, whatever occurred first, death or discharge from ICU. Considering secondary endpoints such as ICU length of stay, an additional observation period of 30 days is scheduled for each patient (figure 2).

Randomization

Block-balanced randomization, in a 1:1 ratio (n = 20 ketogenic enteral nutrition; n = 20 conventional enteral nutrition), is computer-generated by StatsDirect (StatsDirect, Cambridge, UK) with random block sizes between n = 10 and n = 20, additionally using random permutations of treatments within each block. Investigators are blinded to the allocation according to the randomization list until a patient has been included in the study.

Interventional and study-specific procedures

After study inclusion and randomization, the intervention group will receive a nutritional solution with a ketogenic formulation (KetoCal 4:1, Nutricia, Erlangen, Germany) with 0.61g carbohydrates per 100mL. The controls will receive a standard enteral nutritional solution with 17.0g carbohydrates per 100mL (Fresubin HP Energy, Fresenius Kabi, Bad Homburg Deutschland) likewise started after randomization. As soon as the patients are capable of consuming oral food, the intervention group receives special ketogenic drinking solutions and an individually adapted ketogenic diet plan provided by the hospital's kitchen. The control group will receive a standardized wholesome diet according to the hospital's menu.

All patients will be treated with a multimodal intensive care unit concept according to current sepsis guidelines[24] including analgesia and sedation, fluid therapy, lung-protective mechanical ventilation, hemodynamic monitoring and management, anticoagulation as well as antibiotic treatment and appropriate diagnostics. Most clinical, laboratory and demographic data will be collected during routine care and extracted from hospital and ICU electronic health records and merged in a common case report form (see Supplemental material 1). A comprehensive overview of the study-specific measurements, interventions, planned time points, analyses and data collections is depicted in the study flow chart adapted to SPIRIT recommendations (figure 3).

Briefly, study-specific blood sampling is performed on day 1 (day of study inclusion), and day 14 or end of ketogenic diet. Additionally, ketone body concentration in whole blood (included in daily routine laboratory) and in urine samples will be determined daily in both groups.

Study-specific analysis comprise gene expression profiles of extracted T-cells from 15 ml of whole blood collected in tubes containing Lithium Heparin (Sarstedt, Nümbrecht, Germany). Peripheral Blood Monocytic Cells (PMBC) are extracted by Ficoll density gradient centrifugation (Biochrom, Berlin, Germany) according to the manufacturer's instructions. Subsequently, T cells will be extracted by CD4/CD8 microbead separation (Miltenyi, Bergisch-Gladbach, Germany) according to the manufacturer's protocol.

Additionally, 5 ml of whole blood will be drawn into the PAXGene RNA extraction tubes (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions and stored at -20°C until analysis. For analysis of cytokine expression profiles, 3 ml of whole blood will be drawn into TruCulture tubes (Myriad RBM, Austin, USA) and immediately incubated at 37°C for 48 hours according to the manufacturer's

instructions. Afterwards, the supernatant will be aliquoted and stored at -80°C until analysis.

Objectives

The primary endpoint of the study is to assess if an low-carb diet in septic patients can increase the levels of ketone bodies within 14 days.

The secondary objectives will be to compare the intervention group and the control group with regard to the following:

- Safety and feasibility parameters:
    - Serum cholesterol concentration
    - Serum triglyceride concentration
    - Acid base balance (i.e. risk of metabolic acidosis)
    - Serum aspartate transaminase and alanine transaminase activity
    - Bilirubin concentration
    - Blood glucose concentration and insulin requirements
    - Catecholamine and vasopressor requirements
    - Development of the SOFA Score, SAPSII
    - 30-day mortality
    - ICU and hospital length of stay
    - Short form 36 health questionnaire

- Immunologic parameters:
    - mRNA expression profiles in T cells
    - mRNA expression profiles from whole blood (PAXgene®)
    - TruCulture whole blood stimulation (in vitro), subsequent analysis of cytokine secretion via multiplex assay

- CMV / EBV reactivation rate after 7days + 14days

Data collection

The clinical and demographic documentation of the data will be derived from our patient data management system (PDMS) (Dräger ICM, Dräger Medical, Lübeck, Germany). All study-relevant data will be documented in a pseudonymized case report form (Supplemental material 1). Solely the principal investigator of this study has access to the pseudonymization key and is capable to de-identify the study patient in reasonable situations, e.g. due to severe safety concerns. All study relevant data will subsequently be entered in in a central anonymized data source, along with study-specific measurements, for further statistical analysis. Data entered in the study data source will be monitored by an independent clinical research associate and checked for consistency and missing values ensure adequate data quality. This anonymized study data source will be made available along with the publication. All records, subjects' identities and data management will remain confidential with the General Data Protection Regulation (GDPR) of the European Parliament and the Council of the European Union. Furthermore, this protocol was designed following the SPIRIT recommendations for interventional trials (see Supplemental material 2).

Statistical analysis

Since this is a study designed to demonstrate superiority of the primary endpoint, (increase of ketone body levels upon ketogenic enteral nutrition within 14 days), we will perform an intention-to-treat analysis as recommended by the Consolidated Standards of Reporting Trials guidelines.[25] The per-protocol-population will be defined as randomised patients without major protocol deviations, such as non-considerations of exclusion criteria or missing data for the primary endpoint. The per protocol analysis

will also be made available along with the publication as supplementary material as appropriate. Baseline characteristics of all patients will be described per group. Qualitative data will be described as frequencies and percentages. Continuous variables are presented as mean ± standard deviation in case of normal distribution and as median and IQR (25th and 75th percentile) in case of non-normally distributed variables. Continuous variables will be compared using parametric Student's t-test or non-parametric Mann-Whitney U test. Categorical variables will be characterised by numbers with percentages and will be compared using the $\chi 2$ test or a Fisher's exact test. Superiority will be assumed, if the 95% CI for the difference between the means excludes zero or p values are statistically significantly different at an a priori alpha error of less than 0.05. The graphical processing of variables will be performed depending on the measurement level of the variables as histograms, mean value curves with corresponding SD or box whisker plots.

Ethics and dissemination

A manuscript with the results of the study will be published in a peer-reviewed journal. The study has received the following approvals: Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6657-BR). The study (UTN: U1111-1237-2493) was pre-registered (registration date: 08/02/2019) in the German Clinical Trial Register (www.drks.de; DRKS00017710;) prior to the inclusion of the first study patient (first patient in: 01/22/2020). On completion of the trial, the primary study source data will be made public available along with the publication.

Discussion

An increasing number of experimental studies[8 9 10 17 18] revealed that different nutritional regimes can significantly affect immune cell homeostasis and modulate immune functions. Thus, nutritional interventions may provide an interesting cost-effective and easy-to-apply therapeutic approach to attenuate dysregulation of immune responses during sepsis. In particular ketogenic/very low-carb diets have been shown to inhibit overactivated innate immune cells. Such a diet is based on the restriction of carbohydrates to approximately 30 g/day, which leads to the synthesis of BHB by the liver as an alternative energy source. BHB exerts anti-inflammatory effects by inhibiting the NLRP3 inflammasome, thus preventing the release of the proinflammatory cytokines IL-1β and IL-18.[14] Moreover, BHB stimulates the cellular endogenous antioxidant system and increases the efficiency of the electron transport chain.[13] In a ketogenic diet, not only the production of ketones but also the reduction of carbohydrates contributes to the overall anti-inflammatory effects, as high dietary intake of carbohydrates directly activates the inflammasome and increases the formation of Reactive Oxygen Species (ROS),[9,26,27] which further aggravates inflammation.

Overwhelming inflammation and ROS production are considered as crucial maladaptive hallmarks in sepsis that are associated with organ dysfunction and poor outcome.[28 29 30] Currently, state-of-the-art nutrition in critically ill patients contain more than 40% carbohydrates.[6] So far, it is completely unclear whether these nutritional regimes might enhance the immunological derailment of these patients, and whether a ketogenic diet might be an effective tool to ameliorate uncontrolled inflammation during sepsis.

Ketogenic diets are an established and well tolerated clinical tool to control seizure frequencies in patients suffering from epilepsy.[19,20] However, in rare cases, adverse events, such as hypoglycemia, dehydration, electrolyte alteration, metabolic acidosis, as well as gastrointestinal symptoms, including vomiting, constipation, and diarrhea may occur. Frequency of these side effects of a ketogenic diet in critical ill patients, especially septic patients, has not been investigated, yet.

The current study aims at evaluating the feasibility and safety of a ketogenic diet in sepsis patients, In addition, the effects of this nutritional therapy on inflammatory reactions will be assessed.

Outlook

This study tests the safety and practicability of a ketogenic enteral nutritional therapy in a critical care setting in patients with a severe inflammatory disease. Afterwards, larger cohorts and multicentric approaches will be needed to investigate whether ketogenic nutritional therapy represents a potential treatment strategy to improve sepsis outcome.

Trial status

The first patient was randomized in January 22nd, 2020. The inclusion of participants is ongoing and is expected to continue until February 2021.

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List of abbreviations

DRKS - German Clinical Trials Register

ESPEN - European Society for Clinical Nutrition and Metabolism

ICU - Intensive care unit

ATP - Adenosine Triphosphate

bHB - Beta-Hydroxybutyrate

NLRP3 - NLR Family Pyrin Domain Containing 3

SAPS - Simplified Acute Physiology Score

SOFA - Sequential Organ Failure Asessment

BMI - Body Mass Index

SPIRIT - Standard Protocol Items: Recommendations for Interventional Trials

PBMC - Peripheral Blood Mononuclear Cells

CMV - Cytomegalovirus

EBV - Epstein-Barr Virus

PDMS - Patient Data Management System

GDPR - German Data Protection Regulation

UTN - Universal Trial Number

IL-1β - Interleukin 1, beta

IL-18 - Interleukin 18

ROS - Reactive Oxygen Species

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of the Medical Faculty of the Ruhr-Universität Bochum (No.18-6557-BR) and written informed consent or a positive vote of an independent consultant are eligible for study enrolment.

Consent for publication

Not applicable

Availability of data and material

The data of the described study will be available with the publication as supplementary material.

Conflicts of interests

None to declare.

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Author Statement:

Dr. med. Tim Rahmel and Dr. med. Max Hübner: Main authors of this manuscript, written and revised the manuscript, responsible for study conceptualization and statistical analysis plan

Dr. med. Björn Koos: Supported methodical description and laboratory experiments, participated in the design of this study, and revised the manuscript

Dr. med. Alexander Wolf and Katrin-Maria Willemsen: Contributed to study design and conceptualisation of the methodical approach, supports patient recruitment, and revised the manuscript

Dr. med. Gabriele Strauss: Supports data collection and laboratory analysis, participated in the design of this study, and revised the manuscript

David Efflinger: Supports laboratory analysis, participated in the design of this study, and revised the manuscript

Prof. Dr. med. Michael Adamzik: Supports data collection, reviewed the statistical analysis plan, participated in the design of this study, and revised the manuscript

Prof. Dr. rer nat. Dr med. Simone Kreth: Supporting study conceptualization, drafted the design of this study, reviewed the statistical analysis plan, wrote and revised the manuscript.

All authors read and approved the final manuscript.

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Legends

Figure 1: Estimation results for sample sizes that were needed to receive a statistically significant change in the proportion of positive and negative outcomes via a binomial test scenario for various effect sizes (i.e., Cohen's d) and power values. Each curve represents the results for one specific effect size (from left to right: d = 2.14; d = 1.94; d = 1.74; d = 1.54; d = 1.34), where d = 2.0 is usually considered as appropriate effect size in literature.[11] For the assumed relatively low effect size of d = 1.34, α = 0.05, and 1-β = 0.95 in total about 40 patients were needed.

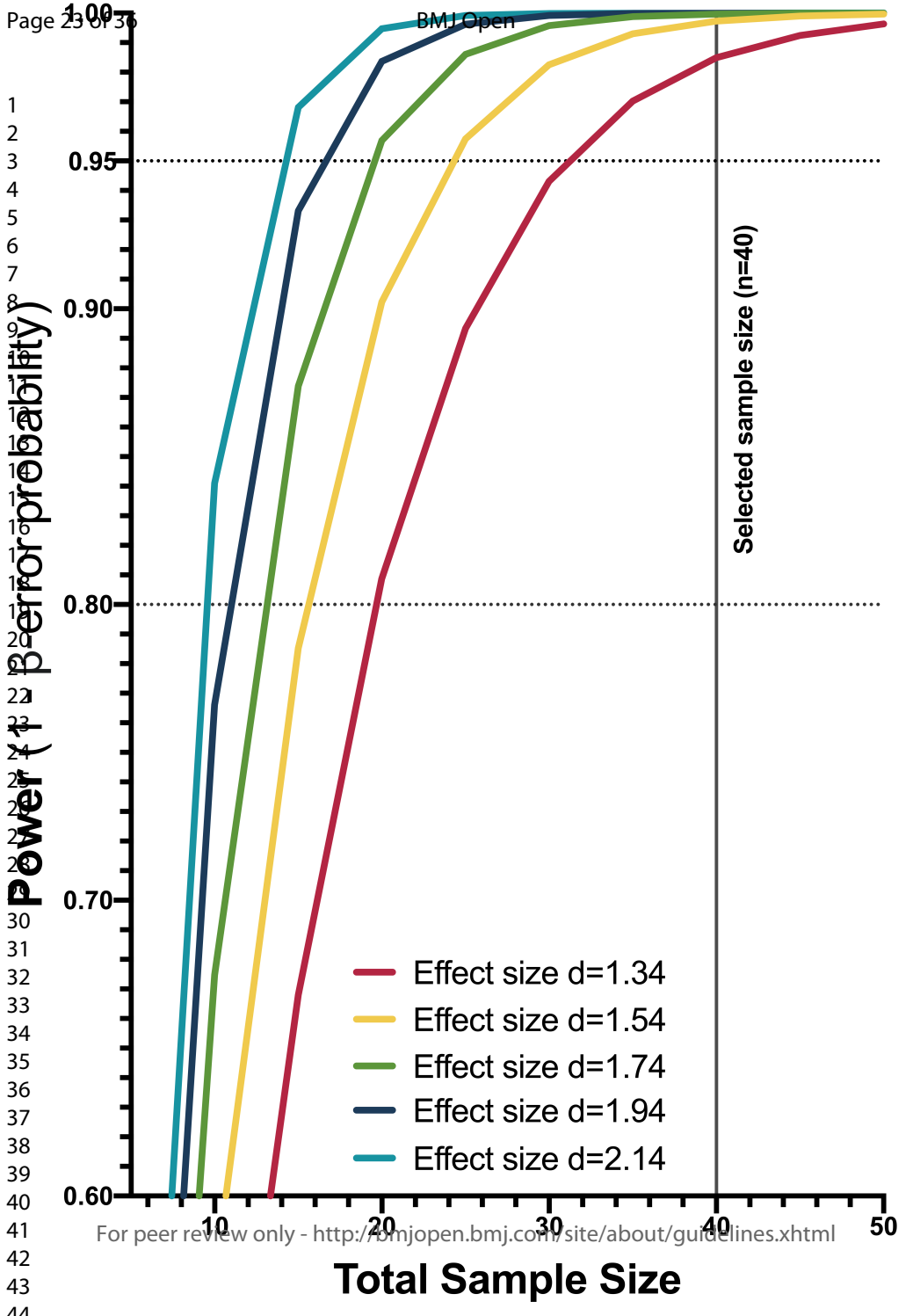
Figure 2: Flowchart of interventional procedures on intervention and control group with duration of each step and performed measurements (RNA = ribonucleic acid tomography; CMV = Cytomegalovirus; ICU = intensive care unit)

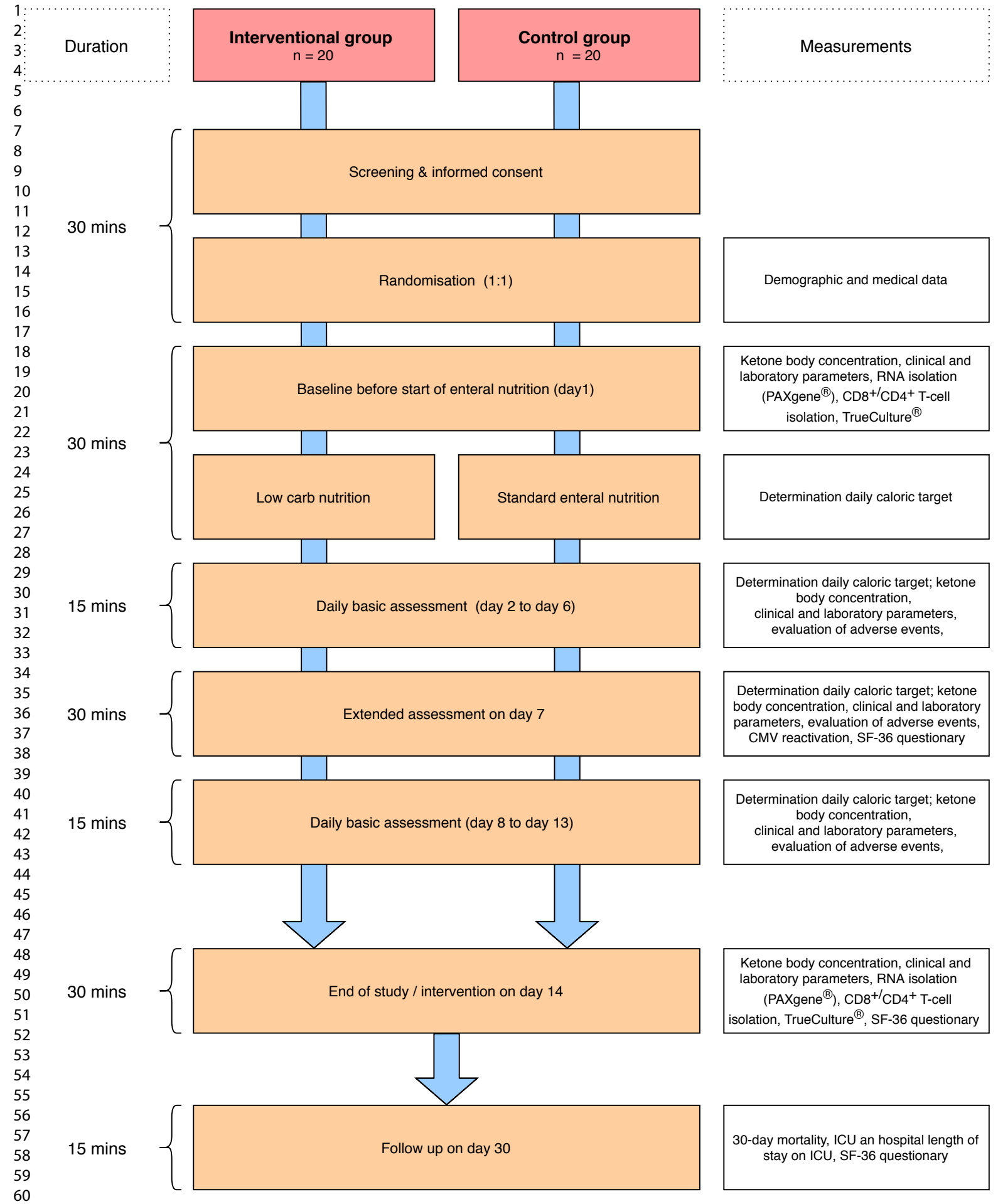
Figure 3: Schedule of enrolment, interventions and assessments – SPIRIT Figure (SPIRIT = Standard Protocol Items: Recommendations for Interventional Trials; RNA = ribonucleic acid)

Supplemental material

Supplemental material 1: Case report form

Supplemental material 2: Spirit checklist




Duration
Interventional group
n = 20
Control group
n = 20
Measurements
Screening & informed consent
30 mins
Randomisation (1:1)
Demographic and medical data
Baseline before start of enteral nutrition (day1)
Ketone body concentration, clinical and laboratory parameters, RNA isolation (PAXgene®), CD8+/CD4+ T-cell isolation, TrueCulture®
30 mins
Low carb nutrition
Standard enteral nutrition
Determination daily caloric target
15 mins
Daily basic assessment (day 2 to day 6)
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events,
30 mins
Extended assessment on day 7
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events, CMV reactivation, SF-36 questionary
15 mins
Daily basic assessment (day 8 to day 13)
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events,
30 mins
End of study / intervention on day 14
Ketone body concentration, clinical and laboratory parameters, RNA isolation (PAXgene®), CD8+/CD4+ T-cell isolation, TrueCulture®, SF-36 questionary
15 mins
Follow up on day 30
30-day mortality, ICU an hospital length of stay on ICU, SF-36 questionary

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation				End of study intervention	Close out
TIMEPOINT	Prior randomisation	Randomisation	Baseline (day 1)	Day 2 to 6	Day 7	Day 8 to 13	Day 14	Day 30
ENROLMENT								
- Eligibility screen	X							
- Informed consent	X							
- Randomisation		X						
STUDY INTERVENTIONS								
- Enteral nutrition			X	X	X	X	X	
ASSESSMENTS								
- Biometrical and demographic data			X					
- Clinical parameter			X	X	X	X	X	
- Ketone body concentration (in blood)			X	X	X	X	X	
- $CD4^+$ and $CD8^+$ T-cell isolation			X				X	
- Whole blood RNA isolation (Pax gene®)			X				X	
- Immunophenotyping (TrueCulture®)			X				X	
- Cytomegalovirus reactivation			X		X		X	
- Questionary "SF 36"			X		X		X	X
- 30-day mortality								X

prob.label version 1.0 |__|__| / |__|__|
patNo. / initials principal investigator

Impact of carbohydrate reduced nutrition in septic patients on ICU - a prospective randomized controlled trial

201__|__|__|.|__|__|.

Inclusion criteria	Yes	No
• age ≥ 18 years	☐	☐
• Diagnosis of sepsis according to current Sepsis-3 definition: 1. Suspected or proven infection 2. Organ dysfunction: increase of SOFA-score ≥ 2scoring points • Inclusion during 36h after diagnosis of sepsis	☐	☐
• Mechanical ventilation <72h		
• Written informed consent or positive vote of an independent consultant	☐	☐

Exclusion criteria	Yes	No
• Refusal of the patient or lack of consent	☐	☐
• Lack of medical indication and/or contraindications to administration of enteral nutrition	☐	☐
• Age < 18 years	☐	☐
• Anaemia (Hb < 8,0g/dl)	☐	☐
• Relationship to the principal investigator (relatives, friends)	☐	☐
• Pre-existing conditions ○ Insulin depended diabetes mellitus type I and II ○ Other severe metabolic disorders or autoimmune disorders ○ Known moderate to severe liver insufficiency or dysfunction ○ Patients with severe refractory metabolic acidosis	☐	☐
• Do not resuscitate order	☐	☐
• Pregnancy or lactation period	☐	☐

- Copy patient documents! (medical history, comorbidity, long term medication, physical examination, ECG, vital signs, etc.) ☐ done
- ☐ male ☐ female height |__|__|__| cm weight |__|__|__|.|__| kg BMI |__|__|.|__| kg/m2
- blood pressure |__|__|__| / |__|__| cardiac frequency |__|__|__| /min temperature |__|__|.|__| °C

- Pregnancy impossible ☐, if possible => see next line

  Pregnancy test (urine) **result**: ☐ neg. ☐ pos. → exclusion

- Note participation in the study in medical record (i.e. PDMS)! ☐ done

- **Studies related documentation**
  - Medical history (space for description):

_____

_____

    - Allergies:

_____

    - Surgeries during the last 5 years:

_____

    - Infective diseases during the last 12 months: YES ☐ / No ☐

  - ICU parameters:

SOFA score (ascertained until day 14 or until release of ICU)

Day 1 _____	day 2 _____	day 3 _____
day 4 _____	day 5 _____	day 6 _____
day 7 _____	day 8 _____	day 9 _____
day 10 _____	day 11 _____	day 12_____
day 13 _____	day 14 _____	

    - Vasopressor therapy (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____	day 2 _____	day 3 _____
day 4 _____	day 5 _____	day 6 _____
day 7 _____	day 8 _____	day 9 _____
day 10 _____	day 11 _____	day 12_____
day 13 _____	day 14 _____	

    - Mechanical ventilation (ascertained until day 14 or until release of ICU)

day 1 _____	day 2 _____	day 3 _____
day 4 _____	day 5 _____	day 6 _____
day 7 _____	day 8 _____	day 9 _____
day 10 _____	day 11 _____	day 12_____
day 13 _____	day 14 _____	

date 201__|__|__|.|__|__| investigator‘s signature _____

- KDIGO-Score (ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Immunosuppression (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Renal dialysis (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Antibiotics therapy (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

date 201__|__|__|.|__|__| investigator‘s signature _____

version1.0

- Secondary infections (yes/no, ascertained until day 14 or until relaese of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Daily print out of routine laboratory investigations (* incl. CMV+EBV-PCR)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7*_____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14*_____

- Daily print out of the vital signs' trend

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Study-related blood sampling

day 1 _____ day 14_____

date 201__|__|__|.|__|__| investigator's signature _____

version1.0

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Impact of carbohydrate reduced nutrition in septic patients on ICU - a prospective randomized controlled trial ***(page 1)***
Trial registration	2a	German trial register (DRKS.de) identifier is DRKS00017710 ***(page 6)***
	2b	Universal Trial Number (UTN) is U1111-1237-2493 ***(page 6)***
Protocol version	3	July 7th, 2019; version 1.1
Funding	4	We acknowledge support by the DFG Open Access Publication Funds of the Ruhr-University Bochum (Ref. No. IN-1214264), just for financial support for publication costs. This will have no impact on our study design or collection, analysis and interpretation of our data. ***(page 19)***

Roles and responsibilities	5a	Dr. med. Tim Rahmel[1] and Dr. med. Max Hübner[2]: Main authors of this manuscript, written and revised the manuscript, responsible for study conceptualization and statistical analysis plan Dr. med. Björn Koos[1]: Supported methodical description and laboratory experiments, participated in the design of this study, and revised the manuscript Dr. med. Alexander Wolf[1] and Katrin-Maria Willemsen[1]: Contributed to study design and conceptualisation of the methodical approach, supports patient recruitment, and revised the manuscript Dr. med. Gabriele Strauss[2]: Supports data collection and laboratory analysis, participated in the design of this study, and revised the manuscript David Efflinger[2]: Supports laboratory analysis, participated in the design of this study, and revised the manuscript Prof. Dr. med. Michael Adamzik[1]: Supports data collection, reviewed the statistical analysis plan, participated in the design of this study, and revised the manuscript Prof. Dr med. Simone Kreth[2]: Supporting study conceptualization, drafted the design of this study, reviewed the statistical analysis plan, wrote and revised the manuscript All authors read and approved the final manuscript. [1] Klinik für Anästhesiologie, Intensivmedizin und Schmerztherapie, Universitätsklinikum Knappschaftskrankenhaus Bochum, D-44892 Bochum, Germany [2] Walter-Brendel Center of Experimental Medicine, Faculty of Medicine, Marchioninistrasse 27, D-81377 München ***(page 20/21)***
	5b	n/a
	5c	We acknowledge support by the DFG Open Access Publication Funds of the Ruhr-University Bochum (Ref. No. IN-1214264), just for financial support for publication costs. This will have no impact on our study design or collection, analysis and interpretation of our data. ***(page 17)***
	5d	n/a

Introduction

Background and rationale	6a	Sepsis is defined as detrimental immune response to an infection. This overwhelming immune reaction often abolishes proper reconstitution of the immune cell homeostasis and in turn increases the risk for further complications. Recent studies suggest a favourable impact of ketone bodies on resolution of inflammation. Thus, a ketogenic diet started within the first days of sepsis may provide a beneficial, easy to apply and cost effective treatment option. Therefore, this study is designed to assess the feasibility, efficiency and safety of a ketogenic diet in septic patients. ***(page 4/5)***

	6b	This trial contributes to assess the feasibility and safety of low carb nutrition compared to standard enteral nutrition (comparator) in septic patients on the intensive care unit. ***(page 6-8)***
Objectives	7	The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days. The secondary objectives will be to compare safety, feasibility and immunologic patterns between the intervention group and the control group. ***(page 10)***
Trial design	8	This study is a randomized, open-label superiority trial, investigating in septic patients regarding the impact of low carb nutrition (intervention) compared to standard nutrition (control). ***(page 6)***

Methods: Participants, interventions, and outcomes

Study setting	9	This study will be conducted at the interdisciplinary, operative intensive care unit (ICU) of University Hospital Knappschaftskrankenhaus Bochum, a university hospital of Ruhr-University Bochum in Bochum, Germany. ***(page 6)***
Eligibility criteria	10	Inclusion criteria are age ≥ 18 years, written informed consent of the patient or their guardian, study enrollment within 36 hours after diagnosis of sepsis, and mechanical ventilation for less than 72 hours on study inclusion. Exclusion criteria are pregnancy or lactation, hemoglobin concentration < 8g/dl, insulin-dependent diabetes, severe and persistently health compromising metabolic disorders or autoimmune diseases, severe liver dysfunction or liver failure, refractory metabolic acidosis, invasive ventilation >72h, diagnosis of sepsis >36h at study enrollment, and contraindications against an enteral nutrition. ***(page 6)***
Interventions	11a	After study inclusion and randomization, the intervention group will receive a low carb nutritional solution (KetoCal 4:1, Nutricia, Erlangen, Germany) with 0.61g carbohydrates per 100mL. The controls will receive a standard enteral nutritional solution with 17.0g carbohydrates per 100mL (Fresubin HP Energy, Fresenius Kabi, Bad Homburg Deutschland) likewise started after randomization. As soon as the patients are capable of consuming oral food, the intervention group receives special ketogenic drinking solutions and also an individually adapted ketogenic diet plan provided by the hospital's kitchen. The control group will receive a standardized wholesome diet according to the common hospital's menu. ***(page 8)***

	11b	Hypoglycaemia, liver failure, metabolic acidosis, and any other kind of suggested severe adverse event, decision of to withdrew from the ketogenic diet ***(page 8)***
	11c	Control of the electronic patient data management system (PDMS) regarding protocol deviations.
	11d	n/a => There are no relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days. The secondary objectives will be to compare safety, feasibility and immunologic patterns between the intervention group and the control group. ***(page 10)***

Participant timeline 13

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation				End of study intervention	Close out
TIMEPOINT	Prior randomisation	Randomisation	Baseline (day 1)	Day 2 to 6	Day 7	Day 8 to 13	Day 14	Day 30
ENROLMENT								
- Eligibility screen	X							
- Informed consent	X							
- Randomisation		X						
STUDY INTERVENTIONS								
- Enteral nutrition			X	X	X	X	X	
ASSESSMENTS								
- Biometrical and demographic data			X					
- Clinical parameter			X	X	X	X	X	
- Ketone body concentration (in blood)			X	X	X	X	X	
- $CD4^+$ and $CD8^+$ T-cell isolation			X				X	
- Whole blood RNA isolation (Pax gene®)			X				X	
- Immunophenotyping (TrueCulture®)			X				X	
- Cytomegalovirus reactivation			X		X		X	
- Questionary "SF 36"			X		X		X	X
- 30-day mortality								X

(see Figure 3)

Sample size 14 In this randomized-controlled study, a total of 40 patients, i.e. 20 patients in the intervention group and 20 patients in the control group, will be enrolled. ***(page 7)***

Recruitment 15 We will ensure patient recruitment by screening patients on ICU daily. Eligible patients will be approached by the principal investigator and/or one of the eligible physicians.

Methods: Assignment of interventions (for controlled trials)

Allocation:		Block-balanced randomization, in a 1:1 ratio, will be computer-generated by StatsDirect (StatsDirect Ltd., Cambridge, United Kingdom) with random block sizes between n = 10 and n = 20, additionally using random permutations of treatments within each block. Investigators will be blinded to the allocation according to the randomization list until the study patient has been included. ***(page 8)***
Sequence generation	16a	Concealment of allocation mechanism will be performed by using sealed envelopes. For each patient included, a sealed envelope will be drawn and opened.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	The block-balanced randomization list will provide trial group allocation sequence.
Blinding (masking)	17a	n/a - no blinding will be performed.
	17b	n/a

Methods: Data collection, management, and analysis

Data collection methods	18a	The documentation of the data will be pseudonymized and computer-assisted from our patient data management system (PDMS) (Dräger ICM, Dräger Medical, Lübeck, Germany) in a central offline database. ***(page 11)***
	18b	All above mentioned parameters will be collected during the patients stay in hospital until discharge, death or 30th day of stay on ICU. In case of discharge from ICU, follow-up to evaluate 30-days survival will be performed by visit on normal ward or a phone call by one of the investigators. ***(page 11)***
Data management	19	All collected data will solely be provided in pseudonymized form for further study analyzation. Access to the pseudonymization key is only available to the principal investigator of this study. ***(page 11)***

Statistical methods	20a	Since this is a study designed to demonstrate superiority of the primary endpoint, whether a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days, we will perform an intention-to-treat analysis as recommended by the Consolidated Standards of Reporting Trials guidelines. The per-protocol population will be defined as randomised patients without major protocol deviations, such as non-considerations of exclusion criteria or missing data for the primary endpoint. The per protocol analysis will also be made available along with the publication as supplementary material as appropriate. Baseline characteristics of all patients will be described per group. Qualitative data will be described as frequencies and percentages. Continuous variables are presented as mean ± standard deviation in case of normal distribution and as median and IQR (25th and 75th percentile) in case of non-normally distributed variables. Continuous variables will be compared using para- metric Student's t-test or non-parametric Mann-Whitney U test. Categorical variables will be characterised by numbers with percentages and will be compared using the $\chi 2$ test or a Fisher's exact test. Superiority will be assumed, if the 95% CI for the difference between the means excludes zero or p values are statistically significantly different at an a priori alpha error of less than 0.05.The graphical processing of variables will be performed depending on the measurement level of the variables as histograms, mean value curves with corresponding SD or box whisker plots. ***(page 11+12)***
	20b	N/A
	20c	We will perform an intention-to-treat and additionally a per-protocol analysis as recommended by the CONSORT guidelines. ***(page 11+12)***
Methods: Monitoring		
Data monitoring	21a	Data entered in the central offline database will be monitored by an independent clinical research associate and checked for consistency and missing values. ***(page 11)***
	21b	No interim analyses are planned.
Harms	22	During study conduct and follow-up patients will be continuously monitored for possible adverse events. Those will be recorded in the database.
Auditing	23	n/a
Ethics and dissemination		
Research ethics approval	24	This study was reviewed and approved by the Ethics Committee of the Medical Faculty of Ruhr-University Bochum (18-6657). ***(page 6)***

Protocol amendments	25	Principal investigator will communicate all important modifications to study personal.
Consent or assent	26a	Informed consent will be obtained by principal investigator and/or eligible physicians. ***(page 6)***
	26b	n/a
Confidentiality	27	All records, subjects' identities and data management will remain confidential with the General Data Protection Regulation (GDPR) of the European Parliament and the Council of the European Union. ***(page 11)***
Declaration of interests	28	None to declare ***(page 19)***
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	n/a
	31c	A publication of this study protocol in BMJ Open is submitted.
Appendices		
Informed consent materials	32	An informed consent form is available in German language can be obtained from the authors.
Biological specimens	33	n/a - all specimens will be discarded after study-related analysis

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Impact of carbohydrate reduced nutrition in septic patients on ICU - study protocol for a prospective randomized controlled trial

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Secondary Subject Heading:	Intensive care
Keywords:	IMMUNOLOGY, INTENSIVE & CRITICAL CARE, NUTRITION & DIETETICS, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

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Impact of carbohydrate reduced nutrition in septic patients on ICU - study protocol for a prospective randomized controlled trial

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Running head: Low carb nutrition in sepsis

Word count: 2767 (Introduction: 453; Methods/Design: 1784; Discussion: 530)

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Abstract

Introduction: Sepsis is defined as detrimental immune response to an infection. This overwhelming reaction often abolishes a normal reconstitution of the immune cell homeostasis that in turn increases the risk for further complications. Recent studies revealed a favorable impact of ketone bodies on resolution of inflammation. Thus, a ketogenic diet may provide an easy-to-apply and cost-effective treatment option potentially alleviating sepsis-evoked harm. This study is designed to assess the feasibility, efficiency and safety of a ketogenic diet in septic patients.

Methods and analysis: This monocentric study is a randomized, controlled, and open-label trial, conducted on an intensive care unit of a German university hospital. As intervention enteral nutrition with reduced amount of carbohydrates (ketogenic) or standard enteral nutrition (control) is applied. The primary endpoint is the detection of ketone bodies in patients' blood and urine samples. As secondary endpoints the impact on important safety relevant issues (e.g. glucose metabolism, lactate serum concentration, incidence of metabolic acidosis, thyroid function, and 30-day mortality) and the effect on the immune system is analysed.

Ethics and dissemination: The study has received the following approvals: Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6557-BR). Results will be made available to critical care survivors, their caregivers, the funders, the critical care societies and other researchers by publication in a peer-reviewed journal.

Trial registration: German trial register (DRKS.de) identifier is DRKS00017710 pre-registered on August 2nd, 2019; Universal Trial Number (UTN) is U1111-1237-2493

Article summary

Strengths and limitations of this study

- This is the first randomized controlled trial assessing the feasibility and safety of a low-carb nutrition in sepsis.
- Based on strong scientific reasoning derived from other patient populations, our secondary endpoints will provide first insights into the immunological impact of a ketogenic diet in critically ill septic patients.
- A strength of this clinical trial is the pragmatic nature as it uses a mainstay of patient care, i.e. nutrition, as intervention with easy applicability in daily clinical care.
- Our controlled and longitudinal study design will allow us to interpret alterations over time in the intervention and control group, and will provide strong evidence for causality.
- A central limitation of this study is the mortality-related loss to follow-up and the resulting missing data points that could impact the internal validity of the results.

Keywords: Sepsis, low carb, ketogenic diet, carbohydrates, nutrition, inflammation

Introduction

Sepsis is a life-threatening condition characterized by a global dysregulation of the immune system: hyperinflammatory reactions, mostly mounted by innate immune cells and immunoparalysis of adaptive immune cells can occur in an unpredictable time course, sequentially or even simultaneously.[1 2 3]

Despite intensive research efforts during the last decade, mortality rates of sepsis still range around 30-50%, and causal therapies reconstituting immune homeostasis are not available so far.[4] In this situation, the impact of nutrition could gain importance, as metabolism has emerged as a major guiding force of immune cell functions.[5]

According to the ESPEN guideline on clinical nutrition in the ICU, patients receive an enteral nutrition consisting of 1,3g of protein/kg body weight/day, 1,5g of lipids/kg body weight/day. Carbohydrate administration in the range of 4-5mg/kg body weight/minute is recommended, and insulin should be administered at blood glucose levels >180mg/dl.[6] This regimen might now be reconsidered as recent experimental studies revealed that high intake of carbohydrates and consecutive secretion of insulin induces pro-inflammatory reactions of innate immune cells.[7] In line with these findings, a number of convincing studies have recently shown that reducing carbohydrate intake significantly stabilizes immune cell homeostasis and improves survival after systemic bacterial infection.[8 9 10] In these studies, the total amount of carbohydrates is reduced to approximately 10% of the overall calorie intake, whereas protein amounts are kept constant and fat amounts are increased.[11 12] The reduced availability of glucose results in increase of fatty acid oxidation with subsequent synthesis of ketone bodies to cover the body's energy demand and to generate sufficient amounts of ATP.[13] This evolutionary conserved mechanism results in the synthesis of beta-hydroxybutyrate (BHB).[14] However, it becomes increasingly clear that BHB also functions as a

signalling molecule by affecting gene expression via epigenetic alterations, protein modifications, and G-Protein-coupled signaling.[15 16] In recent animal studies, BHB displayed strong anti-inflammatory effects by inhibiting the NLRP3 inflammasome and reducing proinflammatory cytokine secretion of innate immune cells, thus contributing to immune cell homeostasis.[14 16 17 18]

Ketogenic/low carb diets are an established clinical tool in patients suffering from epilepsy. Here, they significantly reduce seizure frequencies without displaying significant adverse effects.[19 20] Also, ketogenic/low carb nutritional regimes have recently been investigated in clinical studies enrolling overweight patients with Type II Diabetes[21] and patients suffering from Glioblastoma.[22] These studies reported no adverse side effects, providing additional evidence that ketogenic/low carb diets are feasible and safe.

In this prospective, randomized controlled trial, we want to assess feasibility and safety of a ketogenic diet in ICU patients suffering from sepsis. Moreover, we will investigate whether enteral administration of a low carb/ketogenic diet induces detectable levels of ketone bodies in septic patients, and whether these ketones are able to modulate immune responses during sepsis.

Methods and analysis

This study is a randomized, open-label trial comparing an interventional group supplied with a low-carb diet and a control group supplied with standard enteral nutrition.

Study population and general data acquisition

This study was reviewed and approved by the Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6657-BR) and registered in the German Clinical Trial Register (DRKS00017710; UTN: U1111-1237-2493) prior to the inclusion of the first study patient. The study will be conducted in accordance with the Declaration of Helsinki and German laws and regulations. All patients are admitted to the intensive care unit (ICU) of University Hospital Knappschaftskrankenhaus Bochum and are recruited from January 2020 (first patient in on January 22nd, 2020) up to February 2021. Patients are considered eligible if study enrolment is completed within 36h after diagnosis of sepsis according to the current Sepsis-3 definition.[23]

Inclusion criteria are age ≥ 18 years, written informed consent of the patient or their guardian (see Supplemental material 1), study enrolment within 36 hours after diagnosis of sepsis and mechanical ventilation for less than 72 hours on study inclusion. Exclusion criteria are pregnancy or lactation, haemoglobin concentration < 8g/dl, insulin-dependent diabetes, severe and persistently health-compromising metabolic disorders or autoimmune diseases, severe liver dysfunction or liver failure, refractory metabolic acidosis, invasive ventilation >72h, diagnosis of sepsis >36h at study enrolment and contraindications against an enteral nutrition.

After randomization, patient data collected are depersonalized via pseudonymization. All pseudonymized and deidentified clinical, biometrical and demographic data will be entered into an offline password-protected study database for later analysis. This dataset will include pre-existing illnesses, frequently used

scores such as the Simplified Acute Physiology Score II (SAPS II) or the Sepsis-related Organ Failure Assessment Score (SOFA), Body Mass Index (BMI), need and duration of renal replacement therapy, ventilator configurations, Horowitz-Index (ratio of PaO2/FiO2), vital parameters (e.g. heart rate, blood pressure, peripheral saturation), medications, amount and dosage of vasopressors and blood laboratory parameters.

Patient and public involvement

Patients were not involved in the development of the research question, outcome measures or study design.

Sample size calculation

In this randomized-controlled study, a total of 40 patients, i.e. 20 patients in the intervention group and 20 patients in the control group, will be enrolled. Based on available data on ketogenic diet regimes for healthy individuals referring to the β-hydroxybutyric acid blood concentration[11] and our estimation of a clinical reasonable effect size, we assume an effect size (Cohen's d) between 1.34 and 2.14 as appropriate. Subsequently, we conducted sample size calculations with varying effect sizes between 1.34 and 2.14 at a level of significance of α=0.05. Based on these calculations, considering the most conservative effect size of 1.34 and assuming a drop-out-rate of 25% as a safety margin, a total sample size of n = 40 (n = 20:20) presents as adequate to achieve a power of 95% (figure 1)

Study design

The total duration of the study is planned for 18 months. It will take 12 months for recruitment of patients and collection of data. The last 6 months are scheduled for

analyses. An individual study duration of 14 days is scheduled for each patient (figure 2). This includes study education and randomization (30 minutes), data collection, intervention with accompanying data collection in both interventional and control group (14 days). End of study is reached on day 14 (along with end of intervention) or, whatever occurred first, death or discharge from ICU. Considering secondary endpoints such as ICU length of stay, an additional observation period of 30 days is scheduled for each patient (figure 2).

Randomization

Block-balanced randomization, in a 1:1 ratio (n = 20 ketogenic enteral nutrition; n = 20 conventional enteral nutrition), is computer-generated by StatsDirect (StatsDirect, Cambridge, UK) with random block sizes between n = 10 and n = 20, additionally using random permutations of treatments within each block. Investigators are blinded to the allocation according to the randomization list until a patient has been included in the study.

Interventional and study-specific procedures

After study inclusion and randomization, the intervention group will receive a nutritional solution with a ketogenic formulation (KetoCal 4:1, Nutricia, Erlangen, Germany) with 0.61g carbohydrates per 100mL. The controls will receive a standard enteral nutritional solution with 17.0g carbohydrates per 100mL (Fresubin HP Energy, Fresenius Kabi, Bad Homburg Deutschland) likewise started after randomization. The energy expenditure to determine the daily calorie goal is estimated by using indirect calorimetry (Q-NRG+, COSMED, Rome, Italy). The enteral nutrition is commenced at an initial rate of 20 mL/h, and increased by 20 mL/h every 6 h in the absence of significant gastric residuals (i.e., ≥ 500 mL), with the aim of reaching the estimated

calorie goal within 24 h after study enrolment. The attending physician is responsible for ensuring the achievement of energy targets. The exact calorie intake is electronically recorded and saved in the electronic health records.

As soon as the patients are capable of consuming oral food, the intervention group receives special ketogenic drinking solutions and an individually adapted ketogenic diet plan provided by the hospital's kitchen. The control group will receive a standardized wholesome diet according to the hospital's menu.

All patients will be treated with a multimodal intensive care unit concept according to current sepsis guidelines[24] including analgesia and sedation, fluid therapy, lung-protective mechanical ventilation, hemodynamic monitoring and management, anticoagulation as well as antibiotic treatment and appropriate diagnostics. Most clinical, laboratory and demographic data will be collected during routine care and extracted from hospital and ICU electronic health records and merged in a common case report form (see Supplemental material 2). A comprehensive overview of the study-specific measurements, interventions, planned time points, analyses and data collections is depicted in the study flow chart adapted to SPIRIT recommendations (figure 3).

Briefly, study-specific blood sampling is performed on day 1 (day of study inclusion), and day 14 or end of ketogenic diet. Additionally, ketone body concentration in whole blood (included in daily routine laboratory) and in urine samples will be determined daily in both groups.

Study-specific analysis comprise gene expression profiles of extracted T-cells from 15 ml of whole blood collected in tubes containing Lithium Heparin (Sarstedt, Nümbrecht, Germany). Peripheral Blood Monocytic Cells (PMBC) are extracted by Ficoll density gradient centrifugation (Biochrom, Berlin, Germany) according to the manufacturer's instructions. Subsequently, T cells will be extracted by CD4/CD8

microbead separation (Miltenyi, Bergisch-Gladbach, Germany) according to the manufacturer's protocol.

Additionally, 5 ml of whole blood will be drawn into the PAXGene RNA extraction tubes (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions and stored at -20°C until analysis. For analysis of cytokine expression profiles, 3 ml of whole blood will be drawn into TruCulture tubes (Myriad RBM, Austin, USA) and immediately incubated at 37°C for 48 hours according to the manufacturer's instructions. Afterwards, the supernatant will be aliquoted and stored at -80°C until analysis.

Objectives

The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase hydroxybutyric acid concentration in blood within 14 days.

The secondary objectives will be to compare the intervention group and the control group with regard to the following:

- Safety and feasibility parameters:
    - Serum cholesterol concentration
    - Serum triglyceride concentration
    - Acid base balance (i.e. risk of metabolic acidosis)
    - Serum aspartate transaminase and alanine transaminase activity
    - Bilirubin concentration
    - Blood glucose concentration and insulin requirements
    - Catecholamine and vasopressor requirements
    - Development of the SOFA Score, SAPSII
    - 30-day mortality
    - ICU and hospital length of stay

- Short form 36 health questionnaire

- Immunologic parameters:
  - mRNA expression profiles in T cells
  - mRNA expression profiles from whole blood (PAXgene®)
  - TruCulture whole blood stimulation (in vitro), subsequent analysis of cytokine secretion via multiplex assay
  - CMV / EBV reactivation rate after 7days + 14days

Data collection

The clinical and demographic documentation of the data will be derived from our patient data management system (PDMS) (Dräger ICM, Dräger Medical, Lübeck, Germany). All study-relevant data will be documented in a pseudonymized case report form (Supplemental material 2). Solely the principal investigator of this study has access to the pseudonymization key and is capable to de-identify the study patient in reasonable situations, e.g. due to severe safety concerns. All study relevant data will subsequently be entered in in a central anonymized data source, along with study-specific measurements, for further statistical analysis. Data entered in the study data source will be monitored by an independent clinical research associate and checked for consistency and missing values ensure adequate data quality. This anonymized study data source will be made available along with the publication. All records, subjects' identities and data management will remain confidential with the General Data Protection Regulation (GDPR) of the European Parliament and the Council of the European Union. Furthermore, this protocol was designed following the SPIRIT recommendations for interventional trials (see Supplemental material 3).

Statistical analysis

Since this is a study designed to demonstrate superiority of the primary endpoint, (increase of ketone body levels upon ketogenic enteral nutrition within 14 days), we will perform an intention-to-treat analysis as recommended by the Consolidated Standards of Reporting Trials guidelines.[25] The per-protocol-population will be defined as randomised patients without major protocol deviations, such as non-considerations of exclusion criteria or missing data for the primary endpoint. The per protocol analysis will also be made available along with the publication as supplementary material as appropriate. Baseline characteristics of all patients will be described per group. Qualitative data will be described as frequencies and percentages. Continuous variables are presented as mean ± standard deviation in case of normal distribution and as median and IQR (25th and 75th percentile) in case of non-normally distributed variables. Continuous variables will be compared using parametric Student's t-test or non-parametric Mann-Whitney U test. Categorical variables will be characterised by numbers with percentages and will be compared using the $\chi 2$ test or a Fisher's exact test. Superiority will be assumed, if the 95% CI for the difference between the means excludes zero or p values are statistically significantly different at an a priori alpha error of less than 0.05. The graphical processing of variables will be performed depending on the measurement level of the variables as histograms, mean value curves with corresponding SD or box whisker plots.

Ethics and dissemination

A manuscript with the results of the study will be published in a peer-reviewed journal. The study has received the following approvals: Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6657-BR). The study (UTN: U1111-1237-2493) was pre-registered (registration date: 08/02/2019) in the German Clinical Trial

Register (www.drks.de; DRKS00017710;) prior to the inclusion of the first study patient (first patient in: 01/22/2020). On completion of the trial, the primary study source data will be made public available along with the publication.

Discussion

An increasing number of experimental studies[8 9 10 17 18] revealed that different nutritional regimes can significantly affect immune cell homeostasis and modulate immune functions. Thus, nutritional interventions may provide an interesting cost-effective and easy-to-apply therapeutic approach to attenuate dysregulation of immune responses during sepsis. In particular ketogenic/very low-carb diets have been shown to inhibit overactivated innate immune cells. Such a diet is based on the restriction of carbohydrates to approximately 30 g/day, which leads to the synthesis of BHB by the liver as an alternative energy source. BHB exerts anti-inflammatory effects by inhibiting the NLRP3 inflammasome, thus preventing the release of the proinflammatory cytokines IL-1β and IL-18.[14] Moreover, BHB stimulates the cellular endogenous antioxidant system and increases the efficiency of the electron transport chain.[13] In a ketogenic diet, not only the production of ketones but also the reduction of carbohydrates contributes to the overall anti-inflammatory effects, as high dietary intake of carbohydrates directly activates the inflammasome and increases the formation of Reactive Oxygen Species (ROS),[9 26 27] which further aggravates inflammation.

Overwhelming inflammation and ROS production are considered as crucial maladaptive hallmarks in sepsis that are associated with organ dysfunction and poor outcome.[28 29 30] So far, it is completely unclear whether a ketogenic diet might enhance the immunological derailment of these patients, and whether a low-carb nutrition might be an effective tool to ameliorate uncontrolled inflammation during sepsis.

Currently, state-of-the-art nutrition in critically ill patients contain more than 40% carbohydrates, thus exceeding minimal needs and preventing ketosis.[6] However, the need to provide amounts of glucose above minimal needs in these patients has never

been demonstrated. Furthermore, during a low-carb diet in healthy adults the controlled production of ketone bodies is known to cause a harmless (and potentially even favourable) "substitute" physiological state known as dietary ketosis.[31 32] In this situation, ketone bodies are provided from the liver to extra-hepatic tissues (e.g. CNS) as alternative energetic supply.[13] This spares glucose metabolism via utilisation of ketone bodies as an alternative fuel. Moreover, blood glucose levels remain within the physiological range under low-carb nutrition due to glucogenic sources (glucogenic amino acids and lipolysis-derived glycerol) that are still provided in ketogenic diets.[33]

Ketogenic diets are an established and well tolerated clinical tool to control seizure frequencies in patients suffering from epilepsy.[19 20] However, in rare cases, adverse events, such as hypoglycaemia, dehydration, electrolyte alteration, metabolic acidosis, as well as gastrointestinal symptoms, including vomiting, constipation, and diarrhoea may occur. Frequency of these side effects of a ketogenic diet in critical ill patients, especially septic patients, has not been investigated, yet.

The current study aims at evaluating the feasibility and safety of a ketogenic diet in sepsis patients. In addition, the effects of this nutritional therapy on inflammatory reactions will be assessed.

Outlook

This study tests the safety and practicability of a ketogenic enteral nutritional therapy in a critical care setting in patients with a severe inflammatory disease. Afterwards, larger cohorts and multicentric approaches will be needed to investigate whether ketogenic nutritional therapy represents a potential treatment strategy to improve sepsis outcome.

Trial status

The first patient was randomized in January 22nd, 2020. The inclusion of participants is ongoing and is expected to continue until February 2021.

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List of abbreviations

DRKS - German Clinical Trials Register

ESPEN - European Society for Clinical Nutrition and Metabolism

ICU - Intensive care unit

ATP - Adenosine Triphosphate

bHB - Beta-Hydroxybutyrate

NLRP3 - NLR Family Pyrin Domain Containing 3

SAPS - Simplified Acute Physiology Score

SOFA - Sequential Organ Failure Asessment

BMI - Body Mass Index

SPIRIT - Standard Protocol Items: Recommendations for Interventional Trials

PBMC - Peripheral Blood Mononuclear Cells

CMV - Cytomegalovirus

EBV - Epstein-Barr Virus

PDMS - Patient Data Management System

GDPR - German Data Protection Regulation

UTN - Universal Trial Number

IL-1β - Interleukin 1, beta

IL-18 - Interleukin 18

ROS - Reactive Oxygen Species

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of the Medical Faculty of the Ruhr-Universität Bochum (No.18-6557-BR) and written informed consent or a positive vote of an independent consultant are eligible for study enrolment.

Consent for publication

Not applicable

Availability of data and material

On completion of the trial, the primary study source data will be made public available along with the publication as supplementary material.

Conflicts of interests

None to declare.

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Author Statement:

Dr. med. Tim Rahmel and Dr. med. Max Hübner: Main authors of this manuscript, written and revised the manuscript, responsible for study conceptualization and statistical analysis plan

Dr. med. Björn Koos: Supported methodical description and laboratory experiments, participated in the design of this study, and revised the manuscript

Dr. med. Alexander Wolf and Katrin-Maria Willemsen: Contributed to study design and conceptualisation of the methodical approach, supports patient recruitment, and revised the manuscript

Dr. med. Gabriele Strauss: Supports data collection and laboratory analysis, participated in the design of this study, and revised the manuscript

David Efflinger: Supports laboratory analysis, participated in the design of this study, and revised the manuscript

Prof. Dr. med. Michael Adamzik: Supports data collection, reviewed the statistical analysis plan, participated in the design of this study, and revised the manuscript

Prof. Dr. rer nat. Dr med. Simone Kreth: Supporting study conceptualization, drafted the design of this study, reviewed the statistical analysis plan, wrote and revised the manuscript.

All authors read and approved the final manuscript.

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Legends

Figure 1: Estimation results for sample sizes that were needed to receive a statistically significant change in the proportion of positive and negative outcomes via a binomial test scenario for various effect sizes (i.e., Cohen's d) and power values. Each curve represents the results for one specific effect size (from left to right: d = 2.14; d = 1.94; d = 1.74; d = 1.54; d = 1.34), where d = 2.0 is usually considered as appropriate effect size in literature.[11] For the assumed relatively low effect size of d = 1.34, α = 0.05, and 1-β = 0.95 in total about 40 patients were needed.

Figure 2: Flowchart of interventional procedures on intervention and control group with duration of each step and performed measurements (RNA = ribonucleic acid tomography; CMV = Cytomegalovirus; ICU = intensive care unit)

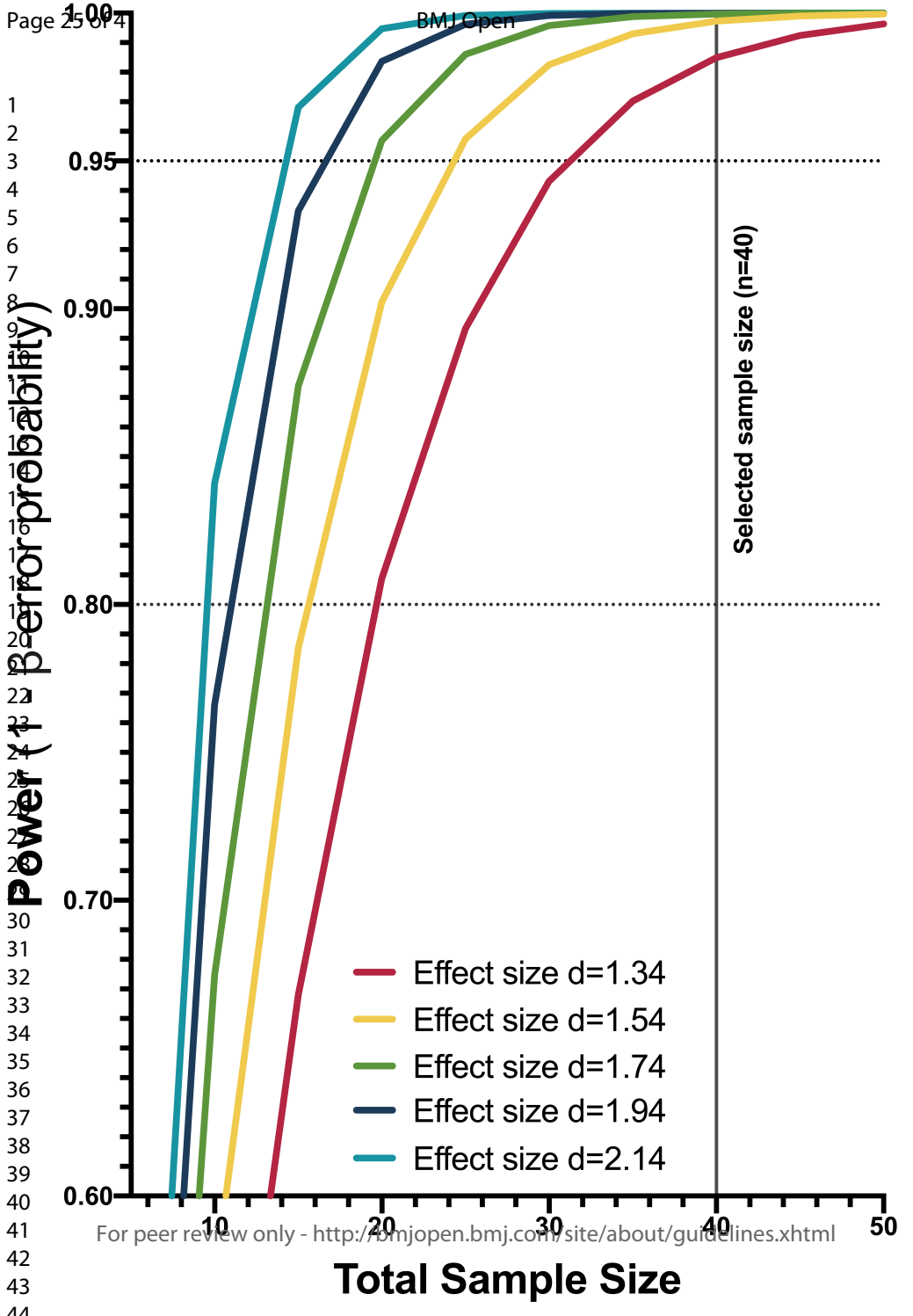
Figure 3: Schedule of enrolment, interventions and assessments – SPIRIT Figure (SPIRIT = Standard Protocol Items: Recommendations for Interventional Trials; RNA = ribonucleic acid)

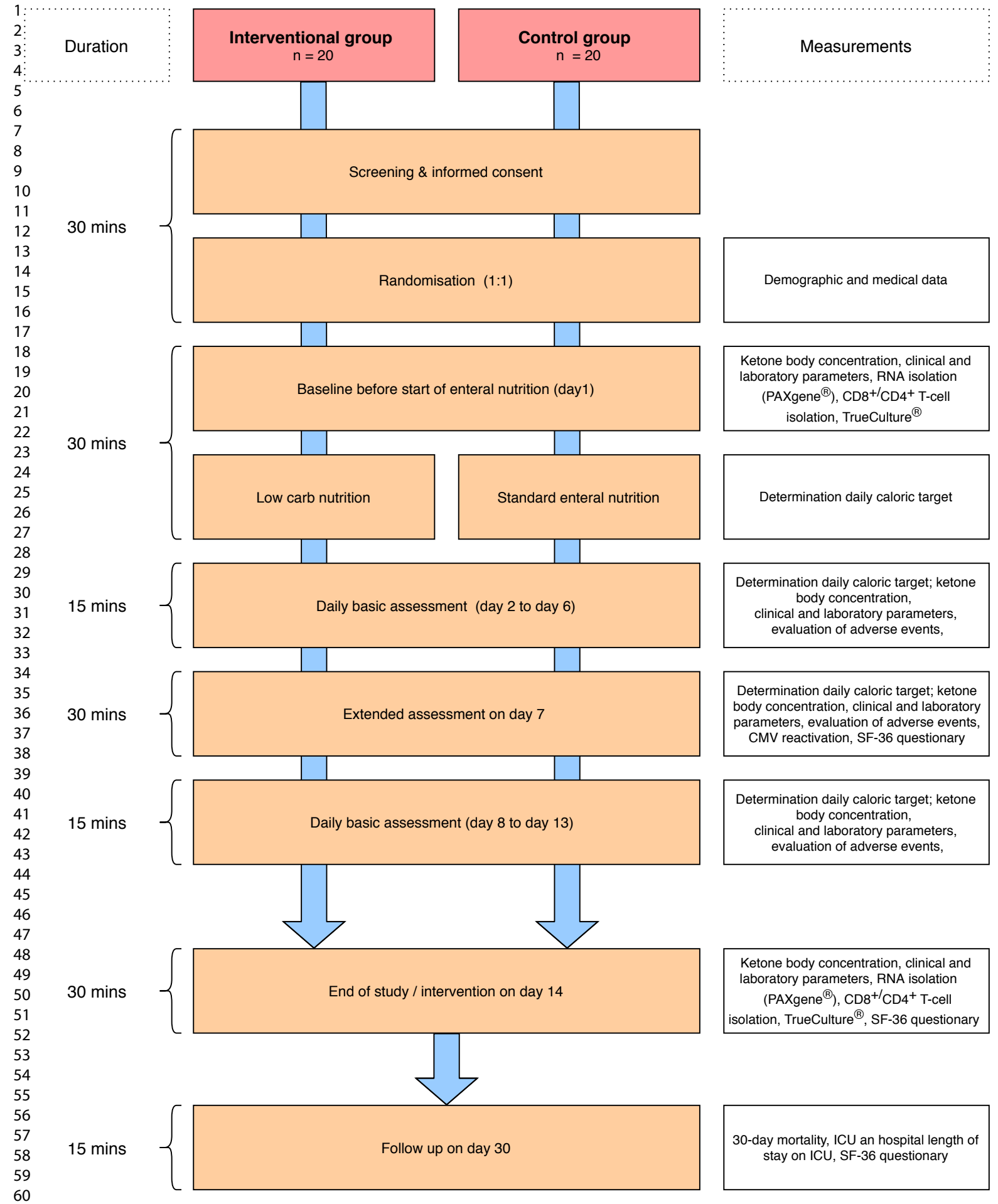
Supplemental material

Supplemental material 1: Informed Consent Form

Supplemental material 2: Case report form

Supplemental material 3: Spirit checklist




Duration
Interventional group
n = 20
Control group
n = 20
Measurements
Screening & informed consent
30 mins
Randomisation (1:1)
Demographic and medical data
Baseline before start of enteral nutrition (day1)
Ketone body concentration, clinical and laboratory parameters, RNA isolation (PAXgene®), CD8+/CD4+ T-cell isolation, TrueCulture®
30 mins
Low carb nutrition
Standard enteral nutrition
Determination daily caloric target
Daily basic assessment (day 2 to day 6)
15 mins
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events,
Extended assessment on day 7
30 mins
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events, CMV reactivation, SF-36 questionary
Daily basic assessment (day 8 to day 13)
15 mins
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events,
End of study / intervention on day 14
30 mins
Ketone body concentration, clinical and laboratory parameters, RNA isolation (PAXgene®), CD8+/CD4+ T-cell isolation, TrueCulture®, SF-36 questionary
Follow up on day 30
15 mins
30-day mortality, ICU an hospital length of stay on ICU, SF-36 questionary

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation				End of study intervention	Close out
TIMEPOINT	Prior randomisation	Randomisation	Baseline (day 1)	Day 2 to 6	Day 7	Day 8 to 13	Day 14	Day 30
ENROLMENT								
- Eligibility screen	X							
- Informed consent	X							
- Randomisation		X						
STUDY INTERVENTIONS								
- Enteral nutrition			X	X	X	X	X	
ASSESSMENTS								
- Biometrical and demographic data			X					
- Clinical parameter			X	X	X	X	X	
- Ketone body concentration (in blood)			X	X	X	X	X	
- $CD4^+$ and $CD8^+$ T-cell isolation			X				X	
- Whole blood RNA isolation (Pax gene®)			X				X	
- Immunophenotyping (TrueCulture®)			X				X	
- Cytomegalovirus reactivation			X		X		X	
- Questionary "SF 36"			X		X		X	X
- 30-day mortality								X

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UK RUB UNIVERSITÄTSKLINIKUM DER RUHR-UNIVERSITÄT BOCHUM

INFORMED CONSENT FORM

Title of the study: **Impact of carbohydrate-reduced nutrition in septic intensive care patients - a prospective randomized controlled trial**

Informing investigator: _____ (Name, Surname)

Study participant: _____ (Name, Surname)

Patient ID: _____

Date of birth: _____ (DD/MM/YYYY)

I hereby confirm that I read and understood the handed-out information sheet (Version 1.3, dated 11th July 2019) for the above-mentioned study, was satisfactory informed by the study investigator, and had the opportunity to ask and clarify all my questions.

I hereby declare my consent to participate in blood sampling and testing according to the provided information. I know that my participation is voluntary and that I can withdraw my consent at any time without giving a reason, without my medical care or legal rights being affected. I can also request the destruction of my blood / tissue sample without any restrictions or disadvantages until analyzation of my samples are completed.

I am aware that I will not financially benefit from this research project (e.g. in terms of study payments) and that the results will not be communicated to me prior to publication. If, contrary to expectations, medical information or findings are obtained from the collected materials that are of significant importance for the maintenance or restoration of my own health, I and my general practitioner will be informed by the study investigators in order to discuss the further procedure with my general practitioner. Thus, I understand that my general practitioner will be informed about my study participation.

If explicitly *not* desired, please check:

( ) I *do not want* my study investigators to inform me or my general practitioner about study participation and potential findings as mentioned above.

I want to allow my blood sample to be used as follows (yes / no):

Immune cells isolation	yes □	no □
Staining of the cells and their components	yes □	no □
Isolation of genetic material	yes □	no □

Privacy Statement:

I am aware that my personal data, in particular medical reports about me, will be collected, saved and evaluated in this research project. The use of the information about my health takes place according to legal regulations and requires the following voluntarily given declaration of consent before participating in the research project (i.e. without the following consent I cannot participate in the research project).

1. I agree that within the scope of this study my personal data, in particular information about my health, will be collected and recorded in a case report form (CRF) and in the electronic patient records of the department of anesthesiology, intensive care medicine and pain therapy.

2. In addition, I agree that authorized and confidential agents and regulatory authorities inspect my personal data, in particular my health data, insofar as this is necessary for the verification of the proper execution of the research project. For this measure, I release the medical examiner from medical confidentiality.

3. I have been informed that I can end participation in the research project at any time. If I withdraw my consent to participate in the research project, I have the right to request that all of my personal data are deleted.

4. In connection with the EU General Data Protection Regulation, which came into force on May 25, 2018, I was explicitly informed of the following issues:
   a) As the person responsible for the data processing in the project is the investigator Dr. med. Tim Rahmel.
   b) I was informed about the data protection officer of the study center including his contact details, which are also noted in the patient information.
   c) I was advised of the right to lodge a complaint with a data protection supervisory authority and the competent data protection supervisory authority has been named to me and is noted in the patient information.
   d) I was advised of my right to receive information (including the provision of a copy free of charge) about the personal data concerned and to request that they be corrected or deleted.

I have received a copy of this declaration of consent. The original remains at the study site.

KNAPPSCHAFT KLINIKEN
UNIVERSITÄTSKLINIKUM
KNAPPSCHAFTSKRANKENHAUS BOCHUM

UK RUB UNIVERSITÄTSKLINIKUM DER
RUHR-UNIVERSITÄT BOCHUM

I have read and understood the patient information (Version 1.3, dated 11th July 2019) for this study. The study investigator (named on the top of the first page) answered all my related questions in detail.

_____

Name of study participant

_____ _____

Place, date Signature of study participant

I conducted the screening interview, informed the study participant about all important aspects and answered all questions related to this study, and obtained the research participant's consent.

_____

Name of the study investigator

_____ _____

Place, date Signature of the study investigator

prob.label version 1.0 |__|__| / |__|__|
patNo. / initials principal investigator

Impact of carbohydrate reduced nutrition in septic patients on ICU - a prospective randomized controlled trial

201__|__|__|.|__|__|.

Inclusion criteria	Yes	No
• age ≥ 18 years	☐	☐
• Diagnosis of sepsis according to current Sepsis-3 definition: 1. Suspected or proven infection 2. Organ dysfunction: increase of SOFA-score ≥ 2scoring points • Inclusion during 36h after diagnosis of sepsis	☐	☐
• Mechanical ventilation <72h		
• Written informed consent or positive vote of an independent consultant	☐	☐

Exclusion criteria	Yes	No
• Refusal of the patient or lack of consent	☐	☐
• Lack of medical indication and/or contraindications to administration of enteral nutrition	☐	☐
• Age < 18 years	☐	☐
• Anaemia (Hb < 8,0g/dl)	☐	☐
• Relationship to the principal investigator (relatives, friends)	☐	☐
• Pre-existing conditions ○ Insulin depended diabetes mellitus type I and II ○ Other severe metabolic disorders or autoimmune disorders ○ Known moderate to severe liver insufficiency or dysfunction ○ Patients with severe refractory metabolic acidosis	☐	☐
• Do not resuscitate order	☐	☐
• Pregnancy or lactation period	☐	☐

- Copy patient documents! (medical history, comorbidity, long term medication, physical examination, ECG, vital signs, etc.) ☐ done
- ☐ male ☐ female height |__|__|__| cm weight |__|__|__|.|__| kg BMI |__|__|.|__| kg/m2
- blood pressure |__|__|__| / |__|__| cardiac frequency |__|__|__| /min temperature |__|__|.|__| °C

- Pregnancy impossible ☐, if possible => see next line

  Pregnancy test (urine) **result**: ☐ neg. ☐ pos. → exclusion

- Note participation in the study in medical record (i.e. PDMS)! ☐ done

- **Studies related documentation**
  - Medical history (space for description):

_____

_____

    - Allergies:

_____

    - Surgeries during the last 5 years:

_____

    - Infective diseases during the last 12 months: YES ☐ / No ☐

  - ICU parameters:

SOFA score (ascertained until day 14 or until release of ICU)

Day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

    - Vasopressor therapy (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

    - Mechanical ventilation (ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

date 201__|__|__|.|__|__| investigator‘s signature _____

- KDIGO-Score (ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Immunosuppression (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Renal dialysis (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Antibiotics therapy (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

date 201__|__|__|.|__|__| investigator‘s signature _____

- Secondary infections (yes/no, ascertained until day 14 or until relaese of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Daily print out of routine laboratory investigations (* incl. CMV+EBV-PCR)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7*_____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14*_____

- Daily print out of the vital signs' trend

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Study-related blood sampling

day 1 _____ day 14_____

date 201__|__|__|.|__|__| investigator's signature _____

version1.0

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Impact of carbohydrate reduced nutrition in septic patients on ICU - a prospective randomized controlled trial ***(page 1)***
Trial registration	2a	German trial register (DRKS.de) identifier is DRKS00017710 ***(page 6)***
	2b	Universal Trial Number (UTN) is U1111-1237-2493 ***(page 6)***
Protocol version	3	July 7th, 2019; version 1.1
Funding	4	We acknowledge support by the DFG Open Access Publication Funds of the Ruhr-University Bochum (Ref. No. IN-1214264), just for financial support for publication costs. This will have no impact on our study design or collection, analysis and interpretation of our data. ***(page 19)***

Roles and responsibilities	5a	Dr. med. Tim Rahmel[1] and Dr. med. Max Hübner[2]: Main authors of this manuscript, written and revised the manuscript, responsible for study conceptualization and statistical analysis plan Dr. med. Björn Koos[1]: Supported methodical description and laboratory experiments, participated in the design of this study, and revised the manuscript Dr. med. Alexander Wolf[1] and Katrin-Maria Willemsen[1]: Contributed to study design and conceptualisation of the methodical approach, supports patient recruitment, and revised the manuscript Dr. med. Gabriele Strauss[2]: Supports data collection and laboratory analysis, participated in the design of this study, and revised the manuscript David Efflinger[2]: Supports laboratory analysis, participated in the design of this study, and revised the manuscript Prof. Dr. med. Michael Adamzik[1]: Supports data collection, reviewed the statistical analysis plan, participated in the design of this study, and revised the manuscript Prof. Dr med. Simone Kreth[2]: Supporting study conceptualization, drafted the design of this study, reviewed the statistical analysis plan, wrote and revised the manuscript All authors read and approved the final manuscript. [1] Klinik für Anästhesiologie, Intensivmedizin und Schmerztherapie, Universitätsklinikum Knappschaftskrankenhaus Bochum, D-44892 Bochum, Germany [2] Walter-Brendel Center of Experimental Medicine, Faculty of Medicine, Marchioninistrasse 27, D-81377 München ***(page 20/21)***
	5b	n/a
	5c	We acknowledge support by the DFG Open Access Publication Funds of the Ruhr-University Bochum (Ref. No. IN-1214264), just for financial support for publication costs. This will have no impact on our study design or collection, analysis and interpretation of our data. ***(page 17)***
	5d	n/a

Introduction

Background and rationale	6a	Sepsis is defined as detrimental immune response to an infection. This overwhelming immune reaction often abolishes proper reconstitution of the immune cell homeostasis and in turn increases the risk for further complications. Recent studies suggest a favourable impact of ketone bodies on resolution of inflammation. Thus, a ketogenic diet started within the first days of sepsis may provide a beneficial, easy to apply and cost effective treatment option. Therefore, this study is designed to assess the feasibility, efficiency and safety of a ketogenic diet in septic patients. ***(page 4/5)***

	6b	This trial contributes to assess the feasibility and safety of low carb nutrition compared to standard enteral nutrition (comparator) in septic patients on the intensive care unit. ***(page 6-8)***
Objectives	7	The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days. The secondary objectives will be to compare safety, feasibility and immunologic patterns between the intervention group and the control group. ***(page 10)***
Trial design	8	This study is a randomized, open-label superiority trial, investigating in septic patients regarding the impact of low carb nutrition (intervention) compared to standard nutrition (control). ***(page 6)***

Methods: Participants, interventions, and outcomes

Study setting	9	This study will be conducted at the interdisciplinary, operative intensive care unit (ICU) of University Hospital Knappschaftskrankenhaus Bochum, a university hospital of Ruhr-University Bochum in Bochum, Germany. ***(page 6)***
Eligibility criteria	10	Inclusion criteria are age ≥ 18 years, written informed consent of the patient or their guardian, study enrollment within 36 hours after diagnosis of sepsis, and mechanical ventilation for less than 72 hours on study inclusion. Exclusion criteria are pregnancy or lactation, hemoglobin concentration < 8g/dl, insulin-dependent diabetes, severe and persistently health compromising metabolic disorders or autoimmune diseases, severe liver dysfunction or liver failure, refractory metabolic acidosis, invasive ventilation >72h, diagnosis of sepsis >36h at study enrollment, and contraindications against an enteral nutrition. ***(page 6)***
Interventions	11a	After study inclusion and randomization, the intervention group will receive a low carb nutritional solution (KetoCal 4:1, Nutricia, Erlangen, Germany) with 0.61g carbohydrates per 100mL. The controls will receive a standard enteral nutritional solution with 17.0g carbohydrates per 100mL (Fresubin HP Energy, Fresenius Kabi, Bad Homburg Deutschland) likewise started after randomization. As soon as the patients are capable of consuming oral food, the intervention group receives special ketogenic drinking solutions and also an individually adapted ketogenic diet plan provided by the hospital's kitchen. The control group will receive a standardized wholesome diet according to the common hospital's menu. ***(page 8)***

	11b	Hypoglycaemia, liver failure, metabolic acidosis, and any other kind of suggested severe adverse event, decision of to withdrew from the ketogenic diet ***(page 8)***
	11c	Control of the electronic patient data management system (PDMS) regarding protocol deviations.
	11d	n/a => There are no relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days. The secondary objectives will be to compare safety, feasibility and immunologic patterns between the intervention group and the control group. ***(page 10)***

- Questionary "SF 36"			X		X		X	X
- 30-day mortality								X

Participant timeline 13

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation				End of study intervention	Close out
TIMEPOINT	Prior randomisation	Randomisation	Baseline (day 1)	Day 2 to 6	Day 7	Day 8 to 13	Day 14	Day 30
ENROLMENT								
- Eligibility screen	X							
- Informed consent	X							
- Randomisation		X						
STUDY INTERVENTIONS								
- Enteral nutrition			X	X	X	X	X	
ASSESSMENTS								
- Biometrical and demographic data			X					
- Clinical parameter			X	X	X	X	X	
- Ketone body concentration (in blood)			X	X	X	X	X	
- $CD4^+$ and $CD8^+$ T-cell isolation			X				X	
- Whole blood RNA isolation (Pax gene®)			X				X	
- Immunophenotyping (TrueCulture®)			X				X	
- Cytomegalovirus reactivation			X		X		X	
- Questionary "SF 36"			X		X		X	X
- 30-day mortality								X

(see Figure 3)

Sample size 14 In this randomized-controlled study, a total of 40 patients, i.e. 20 patients in the intervention group and 20 patients in the control group, will be enrolled. ***(page 7)***

Recruitment 15 We will ensure patient recruitment by screening patients on ICU daily. Eligible patients will be approached by the principal investigator and/or one of the eligible physicians.

Methods: Assignment of interventions (for controlled trials)

Allocation:		Block-balanced randomization, in a 1:1 ratio, will be computer-generated by StatsDirect (StatsDirect Ltd., Cambridge, United Kingdom) with random block sizes between n = 10 and n = 20, additionally using random permutations of treatments within each block. Investigators will be blinded to the allocation according to the randomization list until the study patient has been included. ***(page 8)***
Sequence generation	16a	Concealment of allocation mechanism will be performed by using sealed envelopes. For each patient included, a sealed envelope will be drawn and opened.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	The block-balanced randomization list will provide trial group allocation sequence.
Blinding (masking)	17a	n/a - no blinding will be performed.
	17b	n/a

Methods: Data collection, management, and analysis

Data collection methods	18a	The documentation of the data will be pseudonymized and computer-assisted from our patient data management system (PDMS) (Dräger ICM, Dräger Medical, Lübeck, Germany) in a central offline database. ***(page 11)***
	18b	All above mentioned parameters will be collected during the patients stay in hospital until discharge, death or 30th day of stay on ICU. In case of discharge from ICU, follow-up to evaluate 30-days survival will be performed by visit on normal ward or a phone call by one of the investigators. ***(page 11)***
Data management	19	All collected data will solely be provided in pseudonymized form for further study analyzation. Access to the pseudonymization key is only available to the principal investigator of this study. ***(page 11)***

Statistical methods	20a	Since this is a study designed to demonstrate superiority of the primary endpoint, whether a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days, we will perform an intention-to-treat analysis as recommended by the Consolidated Standards of Reporting Trials guidelines. The per-protocol population will be defined as randomised patients without major protocol deviations, such as non-considerations of exclusion criteria or missing data for the primary endpoint. The per protocol analysis will also be made available along with the publication as supplementary material as appropriate. Baseline characteristics of all patients will be described per group. Qualitative data will be described as frequencies and percentages. Continuous variables are presented as mean ± standard deviation in case of normal distribution and as median and IQR (25th and 75th percentile) in case of non-normally distributed variables. Continuous variables will be compared using para- metric Student's t-test or non-parametric Mann-Whitney U test. Categorical variables will be characterised by numbers with percentages and will be compared using the $\chi 2$ test or a Fisher's exact test. Superiority will be assumed, if the 95% CI for the difference between the means excludes zero or p values are statistically significantly different at an a priori alpha error of less than 0.05.The graphical processing of variables will be performed depending on the measurement level of the variables as histograms, mean value curves with corresponding SD or box whisker plots. ***(page 11+12)***
	20b	N/A
	20c	We will perform an intention-to-treat and additionally a per-protocol analysis as recommended by the CONSORT guidelines. ***(page 11+12)***
Methods: Monitoring		
Data monitoring	21a	Data entered in the central offline database will be monitored by an independent clinical research associate and checked for consistency and missing values. ***(page 11)***
	21b	No interim analyses are planned.
Harms	22	During study conduct and follow-up patients will be continuously monitored for possible adverse events. Those will be recorded in the database.
Auditing	23	n/a
Ethics and dissemination		
Research ethics approval	24	This study was reviewed and approved by the Ethics Committee of the Medical Faculty of Ruhr-University Bochum (18-6657). ***(page 6)***

Protocol amendments	25	Principal investigator will communicate all important modifications to study personal.
Consent or assent	26a	Informed consent will be obtained by principal investigator and/or eligible physicians. ***(page 6)***
	26b	n/a
Confidentiality	27	All records, subjects' identities and data management will remain confidential with the General Data Protection Regulation (GDPR) of the European Parliament and the Council of the European Union. ***(page 11)***
Declaration of interests	28	None to declare ***(page 19)***
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	n/a
	31c	A publication of this study protocol in BMJ Open is submitted.
Appendices		
Informed consent materials	32	An informed consent form is available as translated copy as supplementary material. The original in German language can be obtained from the authors.
Biological specimens	33	n/a - all specimens will be discarded after study-related analysis

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Impact of carbohydrate reduced nutrition in septic patients on ICU - study protocol for a prospective randomized controlled trial

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Keywords:	IMMUNOLOGY, INTENSIVE & CRITICAL CARE, NUTRITION & DIETETICS, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

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Impact of carbohydrate reduced nutrition in septic patients on ICU - study protocol for a prospective randomized controlled trial

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Running head: Low carb nutrition in sepsis

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Abstract

Introduction: Sepsis is defined as detrimental immune response to an infection. This overwhelming reaction often abolishes a normal reconstitution of the immune cell homeostasis that in turn increases the risk for further complications. Recent studies revealed a favorable impact of ketone bodies on resolution of inflammation. Thus, a ketogenic diet may provide an easy-to-apply and cost-effective treatment option potentially alleviating sepsis-evoked harm. This study is designed to assess the feasibility, efficiency and safety of a ketogenic diet in septic patients.

Methods and analysis: This monocentric study is a randomized, controlled, and open-label trial, conducted on an intensive care unit of a German university hospital. As intervention enteral nutrition with reduced amount of carbohydrates (ketogenic) or standard enteral nutrition (control) is applied. The primary endpoint is the detection of ketone bodies in patients' blood and urine samples. As secondary endpoints the impact on important safety relevant issues (e.g. glucose metabolism, lactate serum concentration, incidence of metabolic acidosis, thyroid function, and 30-day mortality) and the effect on the immune system is analysed.

Ethics and dissemination: The study has received the following approvals: Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6557-BR). Results will be made available to critical care survivors, their caregivers, the funders, the critical care societies and other researchers by publication in a peer-reviewed journal.

Trial registration: German trial register (DRKS.de) identifier is DRKS00017710 pre-registered on August 2nd, 2019; Universal Trial Number (UTN) is U1111-1237-2493

Article summary

Strengths and limitations of this study

- This is the first randomized controlled trial assessing the feasibility and safety of a low-carb nutrition in sepsis.
- Based on strong scientific reasoning derived from other patient populations, our secondary endpoints will provide first insights into the immunological impact of a ketogenic diet in critically ill septic patients.
- A strength of this clinical trial is the pragmatic nature as it uses a mainstay of patient care, i.e. nutrition, as intervention with easy applicability in daily clinical care.
- Our controlled and longitudinal study design will allow us to interpret alterations over time in the intervention and control group, and will provide strong evidence for causality.
- A central limitation of this study is the mortality-related loss to follow-up and the resulting missing data points that could impact the internal validity of the results.

Keywords: Sepsis, low carb, ketogenic diet, carbohydrates, nutrition, inflammation

Introduction

Sepsis is a life-threatening condition characterized by a global dysregulation of the immune system: hyperinflammatory reactions, mostly mounted by innate immune cells and immunoparalysis of adaptive immune cells can occur in an unpredictable time course, sequentially or even simultaneously.[1 2 3]

Despite intensive research efforts during the last decade, mortality rates of sepsis still range around 30-50%, and causal therapies reconstituting immune homeostasis are not available so far.[4] In this situation, the impact of nutrition could gain importance, as metabolism has emerged as a major guiding force of immune cell functions.[5]

According to the ESPEN guideline on clinical nutrition in the ICU, patients receive an enteral nutrition consisting of 1,3g of protein/kg body weight/day, 1,5g of lipids/kg body weight/day. Carbohydrate administration in the range of 4-5mg/kg body weight/minute is recommended, and insulin should be administered at blood glucose levels >180mg/dl.[6] This regimen might now be reconsidered as recent experimental studies revealed that high intake of carbohydrates and consecutive secretion of insulin induces pro-inflammatory reactions of innate immune cells.[7] In line with these findings, a number of convincing studies have recently shown that reducing carbohydrate intake significantly stabilizes immune cell homeostasis and improves survival after systemic bacterial infection.[8 9 10] In these studies, the total amount of carbohydrates is reduced to approximately 10% of the overall calorie intake, whereas protein amounts are kept constant and fat amounts are increased.[11 12] The reduced availability of glucose results in increase of fatty acid oxidation with subsequent synthesis of ketone bodies to cover the body's energy demand and to generate sufficient amounts of ATP.[13] This evolutionary conserved mechanism results in the synthesis of beta-hydroxybutyrate (BHB).[14] However, it becomes increasingly clear that BHB also functions as a

signalling molecule by affecting gene expression via epigenetic alterations, protein modifications, and G-Protein-coupled signaling.[15 16] In recent animal studies, BHB displayed strong anti-inflammatory effects by inhibiting the NLRP3 inflammasome and reducing proinflammatory cytokine secretion of innate immune cells, thus contributing to immune cell homeostasis.[14 16 17 18]

Ketogenic/low carb diets are an established clinical tool in patients suffering from epilepsy. Here, they significantly reduce seizure frequencies without displaying significant adverse effects.[19 20] Also, ketogenic/low carb nutritional regimes have recently been investigated in clinical studies enrolling overweight patients with Type II Diabetes[21] and patients suffering from Glioblastoma.[22] These studies reported no adverse side effects, providing additional evidence that ketogenic/low carb diets are feasible and safe.

In this prospective, randomized controlled trial, we want to assess feasibility and safety of a ketogenic diet in ICU patients suffering from sepsis. Moreover, we will investigate whether enteral administration of a low carb/ketogenic diet induces detectable levels of ketone bodies in septic patients, and whether these ketones are able to modulate immune responses during sepsis.

Methods and analysis

This study is a randomized, open-label trial comparing an interventional group supplied with a low-carb diet and a control group supplied with standard enteral nutrition.

Study population and general data acquisition

This study was reviewed and approved by the Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6657-BR) and registered in the German Clinical Trial Register (DRKS00017710; UTN: U1111-1237-2493) prior to the inclusion of the first study patient. The study will be conducted in accordance with the Declaration of Helsinki and German laws and regulations. All patients are admitted to the intensive care unit (ICU) of University Hospital Knappschaftskrankenhaus Bochum and are recruited from January 2020 (first patient in on January 22nd, 2020) up to February 2021. Patients are considered eligible if study enrolment is completed within 36h after diagnosis of sepsis according to the current Sepsis-3 definition.[23]

Inclusion criteria are age ≥ 18 years, written informed consent of the patient or their guardian, study enrolment within 36 hours after diagnosis of sepsis and mechanical ventilation for less than 72 hours on study inclusion. Exclusion criteria are pregnancy or lactation, haemoglobin concentration < 8g/dl, insulin-dependent diabetes, severe and persistently health-compromising metabolic disorders or autoimmune diseases, severe liver dysfunction or liver failure, refractory metabolic acidosis, invasive ventilation >72h, diagnosis of sepsis >36h at study enrolment and contraindications against an enteral nutrition.

After randomization, patient data collected are depersonalized via pseudonymization. All pseudonymized and deidentified clinical, biometrical and demographic data will be entered into an offline password-protected study database for later analysis. This dataset will include pre-existing illnesses, frequently used

scores such as the Simplified Acute Physiology Score II (SAPS II) or the Sepsis-related Organ Failure Assessment Score (SOFA), Body Mass Index (BMI), need and duration of renal replacement therapy, ventilator configurations, Horowitz-Index (ratio of PaO2/FiO2), vital parameters (e.g. heart rate, blood pressure, peripheral saturation), medications, amount and dosage of vasopressors and blood laboratory parameters.

Patient and public involvement

Patients were not involved in the development of the research question, outcome measures or study design.

Sample size calculation

In this randomized-controlled study, a total of 40 patients, i.e. 20 patients in the intervention group and 20 patients in the control group, will be enrolled. Based on available data on ketogenic diet regimes for healthy individuals referring to the β-hydroxybutyric acid blood concentration[11] and our estimation of a clinical reasonable effect size, we assume an effect size (Cohen's d) between 1.34 and 2.14 as appropriate. Subsequently, we conducted sample size calculations with varying effect sizes between 1.34 and 2.14 at a level of significance of α=0.05. Based on these calculations, considering the most conservative effect size of 1.34 and assuming a drop-out-rate of 25% as a safety margin, a total sample size of n = 40 (n = 20:20) presents as adequate to achieve a power of 95% (figure 1)

Study design

The total duration of the study is planned for 18 months. It will take 12 months for recruitment of patients and collection of data. The last 6 months are scheduled for

analyses. An individual study duration of 14 days is scheduled for each patient (figure 2). This includes study education and randomization (30 minutes), data collection, intervention with accompanying data collection in both interventional and control group (14 days). End of study is reached on day 14 (along with end of intervention) or, whatever occurred first, death or discharge from ICU. Considering secondary endpoints such as ICU length of stay, an additional observation period of 30 days is scheduled for each patient (figure 2).

Randomization

Block-balanced randomization, in a 1:1 ratio (n = 20 ketogenic enteral nutrition; n = 20 conventional enteral nutrition), is computer-generated by StatsDirect (StatsDirect, Cambridge, UK) with random block sizes between n = 10 and n = 20, additionally using random permutations of treatments within each block. Investigators are blinded to the allocation according to the randomization list until a patient has been included in the study.

Interventional and study-specific procedures

After study inclusion and randomization, the intervention group will receive a nutritional solution with a ketogenic formulation (KetoCal 4:1, Nutricia, Erlangen, Germany) with 0.61g carbohydrates per 100mL. The controls will receive a standard enteral nutritional solution with 17.0g carbohydrates per 100mL (Fresubin HP Energy, Fresenius Kabi, Bad Homburg Deutschland) likewise started after randomization. The energy expenditure to determine the daily calorie goal is estimated by using indirect calorimetry (Q-NRG+, COSMED, Rome, Italy). The enteral nutrition is commenced at an initial rate of 20 mL/h, and increased by 20 mL/h every 6 h in the absence of significant gastric residuals (i.e., ≥ 500 mL), with the aim of reaching the estimated

calorie goal within 24 h after study enrolment. The attending physician is responsible for ensuring the achievement of energy targets. The exact calorie intake is electronically recorded and saved in the electronic health records.

As soon as the patients are capable of consuming oral food, the intervention group receives special ketogenic drinking solutions and an individually adapted ketogenic diet plan provided by the hospital's kitchen. The control group will receive a standardized wholesome diet according to the hospital's menu.

All patients will be treated with a multimodal intensive care unit concept according to current sepsis guidelines[24] including analgesia and sedation, fluid therapy, lung-protective mechanical ventilation, hemodynamic monitoring and management, anticoagulation as well as antibiotic treatment and appropriate diagnostics. Most clinical, laboratory and demographic data will be collected during routine care and extracted from hospital and ICU electronic health records and merged in a common case report form (see Supplemental material 1). A comprehensive overview of the study-specific measurements, interventions, planned time points, analyses and data collections is depicted in the study flow chart adapted to SPIRIT recommendations (figure 3).

Briefly, study-specific blood sampling is performed on day 1 (day of study inclusion), and day 14 or end of ketogenic diet. Additionally, ketone body concentration in whole blood (included in daily routine laboratory) and in urine samples will be determined daily in both groups.

Study-specific analysis comprise gene expression profiles of extracted T-cells from 15 ml of whole blood collected in tubes containing Lithium Heparin (Sarstedt, Nümbrecht, Germany). Peripheral Blood Monocytic Cells (PMBC) are extracted by Ficoll density gradient centrifugation (Biochrom, Berlin, Germany) according to the manufacturer's instructions. Subsequently, T cells will be extracted by CD4/CD8

microbead separation (Miltenyi, Bergisch-Gladbach, Germany) according to the manufacturer's protocol.

Additionally, 5 ml of whole blood will be drawn into the PAXGene RNA extraction tubes (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions and stored at -20°C until analysis. For analysis of cytokine expression profiles, 3 ml of whole blood will be drawn into TruCulture tubes (Myriad RBM, Austin, USA) and immediately incubated at 37°C for 48 hours according to the manufacturer's instructions. Afterwards, the supernatant will be aliquoted and stored at -80°C until analysis.

Objectives

The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase hydroxybutyric acid concentration in blood within 14 days.

The secondary objectives will be to compare the intervention group and the control group with regard to the following:

- Safety and feasibility parameters:
    - Serum cholesterol concentration
    - Serum triglyceride concentration
    - Acid base balance (i.e. risk of metabolic acidosis)
    - Serum aspartate transaminase and alanine transaminase activity
    - Bilirubin concentration
    - Blood glucose concentration and insulin requirements
    - Catecholamine and vasopressor requirements
    - Development of the SOFA Score, SAPSII
    - 30-day mortality
    - ICU and hospital length of stay

- Short form 36 health questionnaire

- Immunologic parameters:
  - mRNA expression profiles in T cells
  - mRNA expression profiles from whole blood (PAXgene®)
  - TruCulture whole blood stimulation (in vitro), subsequent analysis of cytokine secretion via multiplex assay
  - CMV / EBV reactivation rate after 7days + 14days

Data collection

The clinical and demographic documentation of the data will be derived from our patient data management system (PDMS) (Dräger ICM, Dräger Medical, Lübeck, Germany). All study-relevant data will be documented in a pseudonymized case report form (Supplemental material 1). Solely the principal investigator of this study has access to the pseudonymization key and is capable to de-identify the study patient in reasonable situations, e.g. due to severe safety concerns. All study relevant data will subsequently be entered in in a central anonymized data source, along with study-specific measurements, for further statistical analysis. Data entered in the study data source will be monitored by an independent clinical research associate and checked for consistency and missing values ensure adequate data quality. This anonymized study data source will be made available along with the publication. All records, subjects' identities and data management will remain confidential with the General Data Protection Regulation (GDPR) of the European Parliament and the Council of the European Union. Furthermore, this protocol was designed following the SPIRIT recommendations for interventional trials (see Supplemental material 2).

Statistical analysis

Since this is a study designed to demonstrate superiority of the primary endpoint, (increase of ketone body levels upon ketogenic enteral nutrition within 14 days), we will perform an intention-to-treat analysis as recommended by the Consolidated Standards of Reporting Trials guidelines.[25] The per-protocol-population will be defined as randomised patients without major protocol deviations, such as non-considerations of exclusion criteria or missing data for the primary endpoint. The per protocol analysis will also be made available along with the publication as supplementary material as appropriate. Baseline characteristics of all patients will be described per group. Qualitative data will be described as frequencies and percentages. Continuous variables are presented as mean ± standard deviation in case of normal distribution and as median and IQR (25th and 75th percentile) in case of non-normally distributed variables. Continuous variables will be compared using parametric Student's t-test or non-parametric Mann-Whitney U test. Categorical variables will be characterised by numbers with percentages and will be compared using the $\chi 2$ test or a Fisher's exact test. Superiority will be assumed, if the 95% CI for the difference between the means excludes zero or p values are statistically significantly different at an a priori alpha error of less than 0.05. The graphical processing of variables will be performed depending on the measurement level of the variables as histograms, mean value curves with corresponding SD or box whisker plots.

Ethics and dissemination

A manuscript with the results of the study will be published in a peer-reviewed journal. The study has received the following approvals: Ethics Committee of the Medical Faculty of Ruhr-University Bochum (No. 18-6657-BR). The study (UTN: U1111-1237-2493) was pre-registered (registration date: 08/02/2019) in the German Clinical Trial

Register (www.drks.de; DRKS00017710;) prior to the inclusion of the first study patient (first patient in: 01/22/2020). On completion of the trial, the primary study source data will be made public available along with the publication.

Discussion

An increasing number of experimental studies[8 9 10 17 18] revealed that different nutritional regimes can significantly affect immune cell homeostasis and modulate immune functions. Thus, nutritional interventions may provide an interesting cost-effective and easy-to-apply therapeutic approach to attenuate dysregulation of immune responses during sepsis. In particular ketogenic/very low-carb diets have been shown to inhibit overactivated innate immune cells. Such a diet is based on the restriction of carbohydrates to approximately 30 g/day, which leads to the synthesis of BHB by the liver as an alternative energy source. BHB exerts anti-inflammatory effects by inhibiting the NLRP3 inflammasome, thus preventing the release of the proinflammatory cytokines IL-1β and IL-18.[14] Moreover, BHB stimulates the cellular endogenous antioxidant system and increases the efficiency of the electron transport chain.[13] In a ketogenic diet, not only the production of ketones but also the reduction of carbohydrates contributes to the overall anti-inflammatory effects, as high dietary intake of carbohydrates directly activates the inflammasome and increases the formation of Reactive Oxygen Species (ROS),[9 26 27] which further aggravates inflammation.

Overwhelming inflammation and ROS production are considered as crucial maladaptive hallmarks in sepsis that are associated with organ dysfunction and poor outcome.[28 29 30] So far, it is completely unclear whether a ketogenic diet might enhance the immunological derailment of these patients, and whether a low-carb nutrition might be an effective tool to ameliorate uncontrolled inflammation during sepsis.

Currently, state-of-the-art nutrition in critically ill patients contain more than 40% carbohydrates, thus exceeding minimal needs and preventing ketosis.[6] However, the need to provide amounts of glucose above minimal needs in these patients has never

been demonstrated. Furthermore, during a low-carb diet in healthy adults the controlled production of ketone bodies is known to cause a harmless (and potentially even favourable) "substitute" physiological state known as dietary ketosis.[31 32] In this situation, ketone bodies are provided from the liver to extra-hepatic tissues (e.g. CNS) as alternative energetic supply.[13] This spares glucose metabolism via utilisation of ketone bodies as an alternative fuel. Moreover, blood glucose levels remain within the physiological range under low-carb nutrition due to glucogenic sources (glucogenic amino acids and lipolysis-derived glycerol) that are still provided in ketogenic diets.[33] Furthermore, hyperglycaemia and insulin resistance are more common complications during sepsis suggesting glucose deprivation as subordinate problem.[34]

Ketogenic diets are an established and well tolerated clinical tool to control seizure frequencies in patients suffering from epilepsy.[19 20] However, in rare cases, adverse events, such as hypoglycaemia, dehydration, electrolyte alteration, metabolic acidosis, as well as gastrointestinal symptoms, including vomiting, constipation, and diarrhoea may occur. Frequency of these side effects of a ketogenic diet in critical ill patients, especially septic patients, has not been investigated, yet. An alternative way that likewise could confer the beneficial effects of ketone bodies is the direct supplementation of ketone esters and salts.[35] However it is not clear if the substitution of ketone bodies is capable to mimic all effects of a low-carb nutrition e.g. due to the absence of the metabolic switch.[36]

The current study aims at evaluating the feasibility and safety of a ketogenic diet in sepsis patients. In addition, the effects of this nutritional therapy on inflammatory reactions will be assessed.

Outlook

This study tests the safety and practicability of a ketogenic enteral nutritional therapy in a critical care setting in patients with a severe inflammatory disease. Afterwards, larger cohorts and multicentric approaches will be needed to investigate whether ketogenic nutritional therapy represents a potential treatment strategy to improve sepsis outcome.

Trial status

The first patient was randomized in January 22nd, 2020. The inclusion of participants is ongoing and is expected to continue until February 2021.

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List of abbreviations

DRKS - German Clinical Trials Register

ESPEN - European Society for Clinical Nutrition and Metabolism

ICU - Intensive care unit

ATP - Adenosine Triphosphate

bHB - Beta-Hydroxybutyrate

NLRP3 - NLR Family Pyrin Domain Containing 3

SAPS - Simplified Acute Physiology Score

SOFA - Sequential Organ Failure Asessment

BMI - Body Mass Index

SPIRIT - Standard Protocol Items: Recommendations for Interventional Trials

PBMC - Peripheral Blood Mononuclear Cells

CMV - Cytomegalovirus

EBV - Epstein-Barr Virus

PDMS - Patient Data Management System

GDPR - German Data Protection Regulation

UTN - Universal Trial Number

IL-1β - Interleukin 1, beta

IL-18 - Interleukin 18

ROS - Reactive Oxygen Species

Declarations

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of the Medical Faculty of the Ruhr-Universität Bochum (No.18-6557-BR) and written informed consent or a positive vote of an independent consultant are eligible for study enrolment.

Consent for publication

Not applicable

Availability of data and material

On completion of the trial, the primary study source data will be made public available along with the publication as supplementary material.

Conflicts of interests

None to declare.

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Author Statement:

Dr. med. Tim Rahmel and Dr. med. Max Hübner: Main authors of this manuscript, written and revised the manuscript, responsible for study conceptualization and statistical analysis plan

Dr. med. Björn Koos: Supported methodical description and laboratory experiments, participated in the design of this study, and revised the manuscript

Dr. med. Alexander Wolf and Katrin-Maria Willemsen: Contributed to study design and conceptualisation of the methodical approach, supports patient recruitment, and revised the manuscript

Dr. med. Gabriele Strauss: Supports data collection and laboratory analysis, participated in the design of this study, and revised the manuscript

David Efflinger: Supports laboratory analysis, participated in the design of this study, and revised the manuscript

Prof. Dr. med. Michael Adamzik: Supports data collection, reviewed the statistical analysis plan, participated in the design of this study, and revised the manuscript

Prof. Dr. rer nat. Dr med. Simone Kreth: Supporting study conceptualization, drafted the design of this study, reviewed the statistical analysis plan, wrote and revised the manuscript.

All authors read and approved the final manuscript.

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Legends

Figure 1: Estimation results for sample sizes that were needed to receive a statistically significant change in the proportion of positive and negative outcomes via a binomial test scenario for various effect sizes (i.e., Cohen's d) and power values. Each curve represents the results for one specific effect size (from left to right: d = 2.14; d = 1.94; d = 1.74; d = 1.54; d = 1.34), where d = 2.0 is usually considered as appropriate effect size in literature.[11] For the assumed relatively low effect size of d = 1.34, α = 0.05, and 1-β = 0.95 in total about 40 patients were needed.

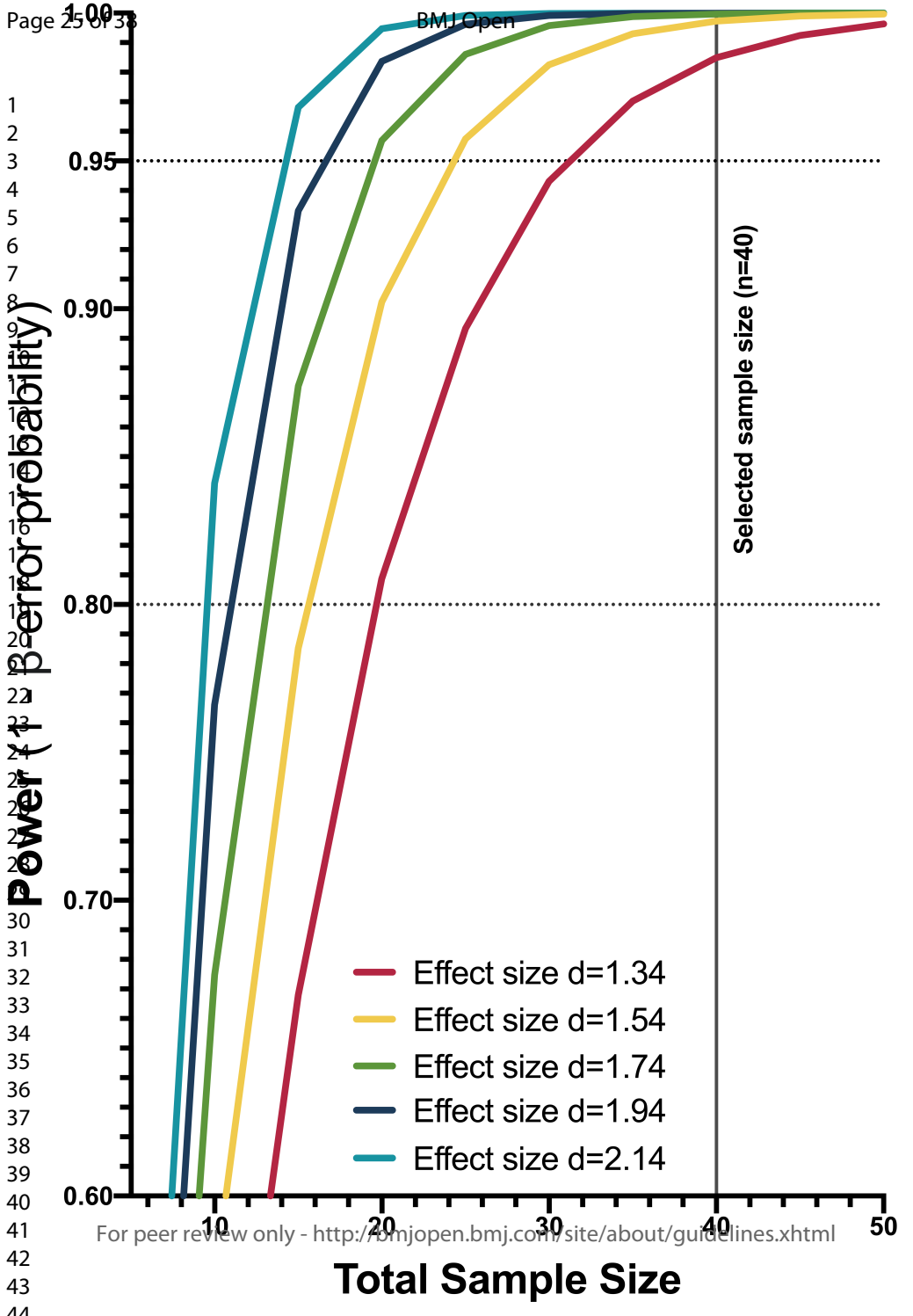
Figure 2: Flowchart of interventional procedures on intervention and control group with duration of each step and performed measurements (RNA = ribonucleic acid tomography; CMV = Cytomegalovirus; ICU = intensive care unit)

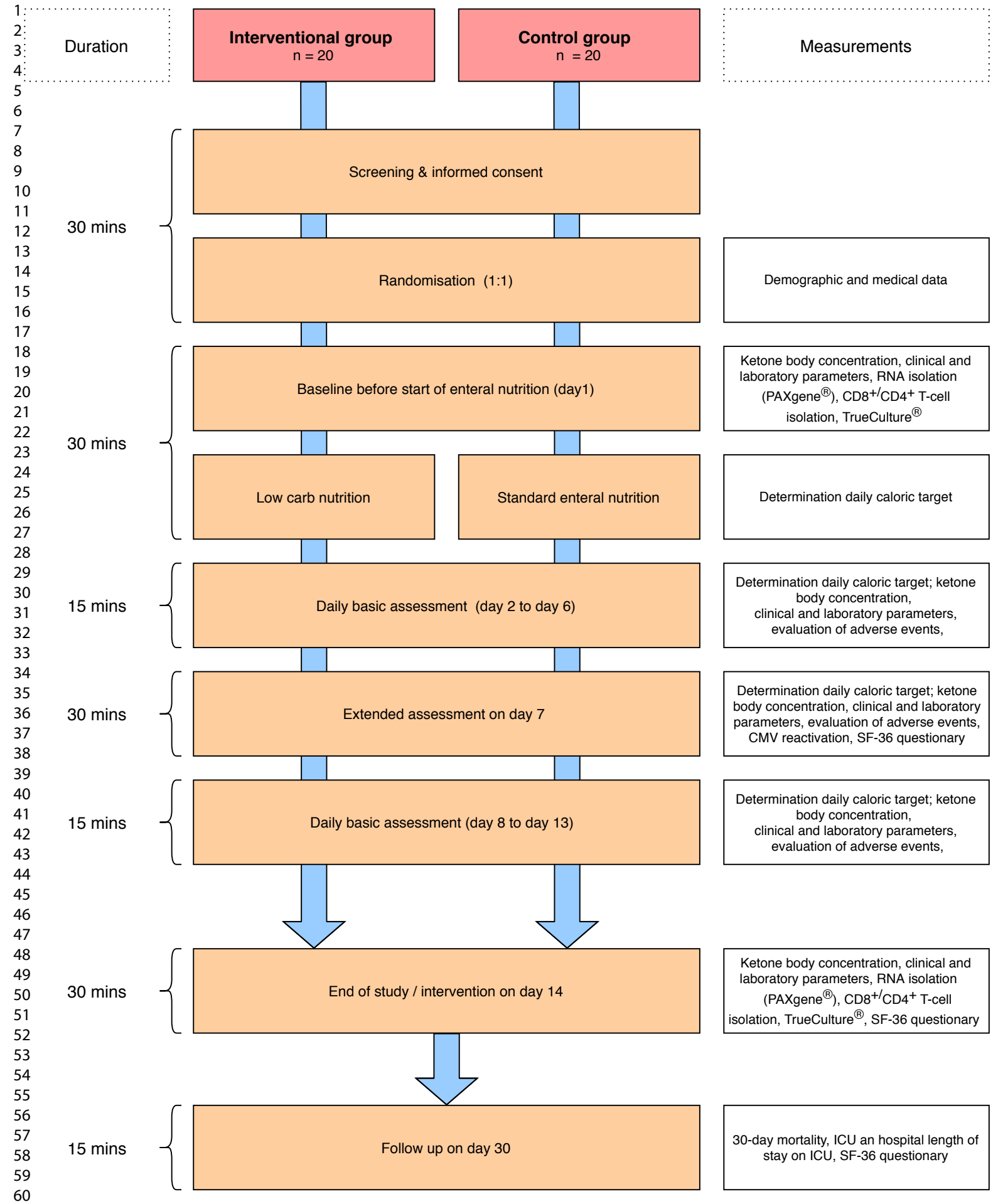
Figure 3: Schedule of enrolment, interventions and assessments – SPIRIT Figure (SPIRIT = Standard Protocol Items: Recommendations for Interventional Trials; RNA = ribonucleic acid)

Supplemental material

Supplemental material 1: Case report form

Supplemental material 2: Spirit checklist




Duration
Interventional group
n = 20
Control group
n = 20
Measurements
Screening & informed consent
30 mins
Randomisation (1:1)
Demographic and medical data
Baseline before start of enteral nutrition (day1)
Ketone body concentration, clinical and laboratory parameters, RNA isolation (PAXgene®), CD8+/CD4+ T-cell isolation, TrueCulture®
30 mins
Low carb nutrition
Standard enteral nutrition
Determination daily caloric target
15 mins
Daily basic assessment (day 2 to day 6)
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events,
30 mins
Extended assessment on day 7
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events, CMV reactivation, SF-36 questionary
15 mins
Daily basic assessment (day 8 to day 13)
Determination daily caloric target; ketone body concentration, clinical and laboratory parameters, evaluation of adverse events,
30 mins
End of study / intervention on day 14
Ketone body concentration, clinical and laboratory parameters, RNA isolation (PAXgene®), CD8+/CD4+ T-cell isolation, TrueCulture®, SF-36 questionary
15 mins
Follow up on day 30
30-day mortality, ICU an hospital length of stay on ICU, SF-36 questionary

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation				End of study intervention	Close out
TIMEPOINT	Prior randomisation	Randomisation	Baseline (day 1)	Day 2 to 6	Day 7	Day 8 to 13	Day 14	Day 30
ENROLMENT								
- Eligibility screen	X							
- Informed consent	X							
- Randomisation		X						
STUDY INTERVENTIONS								
- Enteral nutrition			X	X	X	X	X	
ASSESSMENTS								
- Biometrical and demographic data			X					
- Clinical parameter			X	X	X	X	X	
- Ketone body concentration (in blood)			X	X	X	X	X	
- $CD4^+$ and $CD8^+$ T-cell isolation			X				X	
- Whole blood RNA isolation (Pax gene®)			X				X	
- Immunophenotyping (TrueCulture®)			X				X	
- Cytomegalovirus reactivation			X		X		X	
- Questionary "SF 36"			X		X		X	X
- 30-day mortality								X

prob.label version 1.0 |__|__| / |__|__|
patNo. / initials principal investigator

Impact of carbohydrate reduced nutrition in septic patients on ICU - a prospective randomized controlled trial

201__|__|__|.|__|__|.

Inclusion criteria	Yes	No
• age ≥ 18 years	☐	☐
• Diagnosis of sepsis according to current Sepsis-3 definition: 1. Suspected or proven infection 2. Organ dysfunction: increase of SOFA-score ≥ 2scoring points • Inclusion during 36h after diagnosis of sepsis	☐	☐
• Mechanical ventilation <72h		
• Written informed consent or positive vote of an independent consultant	☐	☐

Exclusion criteria	Yes	No
• Refusal of the patient or lack of consent	☐	☐
• Lack of medical indication and/or contraindications to administration of enteral nutrition	☐	☐
• Age < 18 years	☐	☐
• Anaemia (Hb < 8,0g/dl)	☐	☐
• Relationship to the principal investigator (relatives, friends)	☐	☐
• Pre-existing conditions ○ Insulin depended diabetes mellitus type I and II ○ Other severe metabolic disorders or autoimmune disorders ○ Known moderate to severe liver insufficiency or dysfunction ○ Patients with severe refractory metabolic acidosis	☐	☐
• Do not resuscitate order	☐	☐
• Pregnancy or lactation period	☐	☐

- Copy patient documents! (medical history, comorbidity, long term medication, physical examination, ECG, vital signs, etc.) ☐ done
- ☐ male ☐ female height |__|__|__| cm weight |__|__|__|.|__| kg BMI |__|__|.|__| kg/m2
- blood pressure |__|__|__| / |__|__| cardiac frequency |__|__|__| /min temperature |__|__|.|__| °C

- Pregnancy impossible ☐, if possible => see next line

  Pregnancy test (urine) **result**: ☐ neg. ☐ pos. → exclusion

- Note participation in the study in medical record (i.e. PDMS)! ☐ done

- **Studies related documentation**
  - Medical history (space for description):

_____

_____

    - Allergies:

_____

    - Surgeries during the last 5 years:

_____

    - Infective diseases during the last 12 months: YES ☐ / No ☐

  - ICU parameters:

SOFA score (ascertained until day 14 or until release of ICU)

Day 1 _____	day 2 _____	day 3 _____
day 4 _____	day 5 _____	day 6 _____
day 7 _____	day 8 _____	day 9 _____
day 10 _____	day 11 _____	day 12_____
day 13 _____	day 14 _____	

    - Vasopressor therapy (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____	day 2 _____	day 3 _____
day 4 _____	day 5 _____	day 6 _____
day 7 _____	day 8 _____	day 9 _____
day 10 _____	day 11 _____	day 12_____
day 13 _____	day 14 _____	

    - Mechanical ventilation (ascertained until day 14 or until release of ICU)

day 1 _____	day 2 _____	day 3 _____
day 4 _____	day 5 _____	day 6 _____
day 7 _____	day 8 _____	day 9 _____
day 10 _____	day 11 _____	day 12_____
day 13 _____	day 14 _____	

date 201__|__|__|.|__|__| investigator‘s signature _____

- KDIGO-Score (ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Immunosuppression (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Renal dialysis (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Antibiotics therapy (yes/no, ascertained until day 14 or until release of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

date 201__|__|__|.|__|__| investigator‘s signature _____

- Secondary infections (yes/no, ascertained until day 14 or until relaese of ICU)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Daily print out of routine laboratory investigations (* incl. CMV+EBV-PCR)

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7*_____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14*_____

- Daily print out of the vital signs' trend

day 1 _____ day 2 _____ day 3 _____

day 4 _____ day 5 _____ day 6 _____

day 7 _____ day 8 _____ day 9 _____

day 10 _____ day 11 _____ day 12_____

day 13 _____ day 14 _____

- Study-related blood sampling

day 1 _____ day 14_____

date 201__|__|__|.|__|__| investigator's signature _____

version1.0

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Impact of carbohydrate reduced nutrition in septic patients on ICU - a prospective randomized controlled trial ***(page 1)***
Trial registration	2a	German trial register (DRKS.de) identifier is DRKS00017710 ***(page 6)***
	2b	Universal Trial Number (UTN) is U1111-1237-2493 ***(page 6)***
Protocol version	3	July 7th, 2019; version 1.1
Funding	4	We acknowledge support by the DFG Open Access Publication Funds of the Ruhr-University Bochum (Ref. No. IN-1214264), just for financial support for publication costs. This will have no impact on our study design or collection, analysis and interpretation of our data. ***(page 19)***

Roles and responsibilities	5a	Dr. med. Tim Rahmel[1] and Dr. med. Max Hübner[2]: Main authors of this manuscript, written and revised the manuscript, responsible for study conceptualization and statistical analysis plan Dr. med. Björn Koos[1]: Supported methodical description and laboratory experiments, participated in the design of this study, and revised the manuscript Dr. med. Alexander Wolf[1] and Katrin-Maria Willemsen[1]: Contributed to study design and conceptualisation of the methodical approach, supports patient recruitment, and revised the manuscript Dr. med. Gabriele Strauss[2]: Supports data collection and laboratory analysis, participated in the design of this study, and revised the manuscript David Efflinger[2]: Supports laboratory analysis, participated in the design of this study, and revised the manuscript Prof. Dr. med. Michael Adamzik[1]: Supports data collection, reviewed the statistical analysis plan, participated in the design of this study, and revised the manuscript Prof. Dr med. Simone Kreth[2]: Supporting study conceptualization, drafted the design of this study, reviewed the statistical analysis plan, wrote and revised the manuscript All authors read and approved the final manuscript. [1] Klinik für Anästhesiologie, Intensivmedizin und Schmerztherapie, Universitätsklinikum Knappschaftskrankenhaus Bochum, D-44892 Bochum, Germany [2] Walter-Brendel Center of Experimental Medicine, Faculty of Medicine, Marchioninistrasse 27, D-81377 München ***(page 20/21)***
	5b	n/a
	5c	We acknowledge support by the DFG Open Access Publication Funds of the Ruhr-University Bochum (Ref. No. IN-1214264), just for financial support for publication costs. This will have no impact on our study design or collection, analysis and interpretation of our data. ***(page 17)***
	5d	n/a

Introduction

Background and rationale	6a	Sepsis is defined as detrimental immune response to an infection. This overwhelming immune reaction often abolishes proper reconstitution of the immune cell homeostasis and in turn increases the risk for further complications. Recent studies suggest a favourable impact of ketone bodies on resolution of inflammation. Thus, a ketogenic diet started within the first days of sepsis may provide a beneficial, easy to apply and cost effective treatment option. Therefore, this study is designed to assess the feasibility, efficiency and safety of a ketogenic diet in septic patients. ***(page 4/5)***

	6b	This trial contributes to assess the feasibility and safety of low carb nutrition compared to standard enteral nutrition (comparator) in septic patients on the intensive care unit. ***(page 6-8)***
Objectives	7	The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days. The secondary objectives will be to compare safety, feasibility and immunologic patterns between the intervention group and the control group. ***(page 10)***
Trial design	8	This study is a randomized, open-label superiority trial, investigating in septic patients regarding the impact of low carb nutrition (intervention) compared to standard nutrition (control). ***(page 6)***

Methods: Participants, interventions, and outcomes

Study setting	9	This study will be conducted at the interdisciplinary, operative intensive care unit (ICU) of University Hospital Knappschaftskrankenhaus Bochum, a university hospital of Ruhr-University Bochum in Bochum, Germany. ***(page 6)***
Eligibility criteria	10	Inclusion criteria are age ≥ 18 years, written informed consent of the patient or their guardian, study enrollment within 36 hours after diagnosis of sepsis, and mechanical ventilation for less than 72 hours on study inclusion. Exclusion criteria are pregnancy or lactation, hemoglobin concentration < 8g/dl, insulin-dependent diabetes, severe and persistently health compromising metabolic disorders or autoimmune diseases, severe liver dysfunction or liver failure, refractory metabolic acidosis, invasive ventilation >72h, diagnosis of sepsis >36h at study enrollment, and contraindications against an enteral nutrition. ***(page 6)***
Interventions	11a	After study inclusion and randomization, the intervention group will receive a low carb nutritional solution (KetoCal 4:1, Nutricia, Erlangen, Germany) with 0.61g carbohydrates per 100mL. The controls will receive a standard enteral nutritional solution with 17.0g carbohydrates per 100mL (Fresubin HP Energy, Fresenius Kabi, Bad Homburg Deutschland) likewise started after randomization. As soon as the patients are capable of consuming oral food, the intervention group receives special ketogenic drinking solutions and also an individually adapted ketogenic diet plan provided by the hospital's kitchen. The control group will receive a standardized wholesome diet according to the common hospital's menu. ***(page 8)***

	11b	Hypoglycaemia, liver failure, metabolic acidosis, and any other kind of suggested severe adverse event, decision of to withdrew from the ketogenic diet ***(page 8)***
	11c	Control of the electronic patient data management system (PDMS) regarding protocol deviations.
	11d	n/a => There are no relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	The primary endpoint of the study is to assess if a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days. The secondary objectives will be to compare safety, feasibility and immunologic patterns between the intervention group and the control group. ***(page 10)***

- Questionary "SF 36"			X		X		X	X
- 30-day mortality								X

Participant timeline 13

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation				End of study intervention	Close out
TIMEPOINT	Prior randomisation	Randomisation	Baseline (day 1)	Day 2 to 6	Day 7	Day 8 to 13	Day 14	Day 30
ENROLMENT								
- Eligibility screen	X							
- Informed consent	X							
- Randomisation		X						
STUDY INTERVENTIONS								
- Enteral nutrition			X	X	X	X	X	
ASSESSMENTS								
- Biometrical and demographic data			X					
- Clinical parameter			X	X	X	X	X	
- Ketone body concentration (in blood)			X	X	X	X	X	
- $CD4^+$ and $CD8^+$ T-cell isolation			X				X	
- Whole blood RNA isolation (Pax gene®)			X				X	
- Immunophenotyping (TrueCulture®)			X				X	
- Cytomegalovirus reactivation			X		X		X	
- Questionary "SF 36"			X		X		X	X
- 30-day mortality								X

(see Figure 3)

Sample size 14 In this randomized-controlled study, a total of 40 patients, i.e. 20 patients in the intervention group and 20 patients in the control group, will be enrolled. ***(page 7)***

Recruitment 15 We will ensure patient recruitment by screening patients on ICU daily. Eligible patients will be approached by the principal investigator and/or one of the eligible physicians.

Methods: Assignment of interventions (for controlled trials)

Allocation:		Block-balanced randomization, in a 1:1 ratio, will be computer-generated by StatsDirect (StatsDirect Ltd., Cambridge, United Kingdom) with random block sizes between n = 10 and n = 20, additionally using random permutations of treatments within each block. Investigators will be blinded to the allocation according to the randomization list until the study patient has been included. ***(page 8)***
Sequence generation	16a	Concealment of allocation mechanism will be performed by using sealed envelopes. For each patient included, a sealed envelope will be drawn and opened.
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	The block-balanced randomization list will provide trial group allocation sequence.
Blinding (masking)	17a	n/a - no blinding will be performed.
	17b	n/a

Methods: Data collection, management, and analysis

Data collection methods	18a	The documentation of the data will be pseudonymized and computer-assisted from our patient data management system (PDMS) (Dräger ICM, Dräger Medical, Lübeck, Germany) in a central offline database. ***(page 11)***
	18b	All above mentioned parameters will be collected during the patients stay in hospital until discharge, death or 30th day of stay on ICU. In case of discharge from ICU, follow-up to evaluate 30-days survival will be performed by visit on normal ward or a phone call by one of the investigators. ***(page 11)***
Data management	19	All collected data will solely be provided in pseudonymized form for further study analyzation. Access to the pseudonymization key is only available to the principal investigator of this study. ***(page 11)***

Statistical methods	20a	Since this is a study designed to demonstrate superiority of the primary endpoint, whether a low-carb diet in septic patients can increase the levels of ketone bodies within 14 days, we will perform an intention-to-treat analysis as recommended by the Consolidated Standards of Reporting Trials guidelines. The per-protocol population will be defined as randomised patients without major protocol deviations, such as non-considerations of exclusion criteria or missing data for the primary endpoint. The per protocol analysis will also be made available along with the publication as supplementary material as appropriate. Baseline characteristics of all patients will be described per group. Qualitative data will be described as frequencies and percentages. Continuous variables are presented as mean ± standard deviation in case of normal distribution and as median and IQR (25th and 75th percentile) in case of non-normally distributed variables. Continuous variables will be compared using para- metric Student's t-test or non-parametric Mann-Whitney U test. Categorical variables will be characterised by numbers with percentages and will be compared using the $\chi 2$ test or a Fisher's exact test. Superiority will be assumed, if the 95% CI for the difference between the means excludes zero or p values are statistically significantly different at an a priori alpha error of less than 0.05.The graphical processing of variables will be performed depending on the measurement level of the variables as histograms, mean value curves with corresponding SD or box whisker plots. ***(page 11+12)***
	20b	N/A
	20c	We will perform an intention-to-treat and additionally a per-protocol analysis as recommended by the CONSORT guidelines. ***(page 11+12)***
Methods: Monitoring		
Data monitoring	21a	Data entered in the central offline database will be monitored by an independent clinical research associate and checked for consistency and missing values. ***(page 11)***
	21b	No interim analyses are planned.
Harms	22	During study conduct and follow-up patients will be continuously monitored for possible adverse events. Those will be recorded in the database.
Auditing	23	n/a
Ethics and dissemination		
Research ethics approval	24	This study was reviewed and approved by the Ethics Committee of the Medical Faculty of Ruhr-University Bochum (18-6657). ***(page 6)***

Protocol amendments	25	Principal investigator will communicate all important modifications to study personal.
Consent or assent	26a	Informed consent will be obtained by principal investigator and/or eligible physicians. ***(page 6)***
	26b	n/a
Confidentiality	27	All records, subjects' identities and data management will remain confidential with the General Data Protection Regulation (GDPR) of the European Parliament and the Council of the European Union. ***(page 11)***
Declaration of interests	28	None to declare ***(page 19)***
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	n/a
	31c	A publication of this study protocol in BMJ Open is submitted.
Appendices		
Informed consent materials	32	An informed consent form is available as translated copy as supplementary material. The original in German language can be obtained from the authors.
Biological specimens	33	n/a - all specimens will be discarded after study-related analysis

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.